THE AMERICAN JOURNAL OF PHYSIOLOGY

EDITED FOR

THE AMERICAN PHYSIOLOGICAL SOCIETY

CONTENTS

VOL. LIV—NO. 3

Issued January 1, 1921

BALTIMORE, U. S. A.

1921

Entered as second class matter, August 18, 1914, at the Post Office at Baltimore, Md., under the act of March 3, 1879. Acceptance for mailing at special rate of postage provided for in Section 1103, Act of October 3, 1917. Authorized on July 5, 1918

THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 54 JANUARY 1, 1921 No. 3

EXPERIMENTAL STUDIES IN DIABETES

SERIES II. THE INTERNAL PANCREATIC FUNCTION IN RELATION TO BODY MASS AND METABOLISM

7. The Influence of Cold

FREDERICK M. ALLEN

From the Hospital of The Rockefeller Institute for Medical Research, New York

Received for publication August 10, 1920

According to some earlier literature (1), cold causes hyperglycemia and glycosuria in both warm- and cold-blooded animals, and increases the glycosuria of diabetic dogs. The use of cold and shivering to drive out glycogen from phlorizinized animals was familiarized by Lusk. A single mild test of cold environment in a human patient gave a negative result (2).

A few words may be devoted to the theory of the subject. In a totally phlorizinized dog, it is obvious that a release of carbohydrate which cannot be utilized will result in a temporary rise of glycosuria and of the D : N ratio. A similar mobilization of stored carbohydrate, together with a possible diuretic action of cold, might produce a similar result in a totally depancreatized animal. The extra heat required to maintain body temperature would supposedly be furnished by fat, and there is no reason to expect any increased output of sugar derived from protein, either in the sense of any appreciable increase of protein decomposition or any genuine alteration of the D : N ratio. In a partially depancreatized animal, the increased carbohydrate mobilization or increased total metabolism may impose an additional burden upon the pancreas remnant, and any lasting increase of glycosuria must be interpreted in the sense of a true aggravation of the diabetes. At the same time complicating factors come into play. If the animal

fasts, the extra sugar loss and fat combustion impose a sharper under-nutrition treatment in a cold than in a warm environment. Likewise a diet which is adequate in warmth becomes under-nutrition in the cold. On the other hand an increase of diet to meet the increased requirement complicates the problem still further. Shivering is a form of muscular exercise, and may possibly have a metabolic effect opposed to the effect of cold *per se*.

Normal dogs can live without artificial heat in the ordinary winter weather of New York if merely sheltered, but their metabolism evidently is considerably higher than that of dogs kept in warm rooms. The first experiments were therefore planned to determine whether there is any practical difference in the ease of producing diabetes in dogs kept in outdoor cages in winter and in others kept in a room specially warmed to a high summer temperature. This method was considered better for the purpose than the plan of using severely diabetic dogs and following the variations in their glycosuria, because it was important to distinguish a tendency to the production of mere glycosuria and hyperglycemia (such as cold may excite even in normal animals, which are certainly not diabetic from this cause) from a tendency to the production of actual diabetes. If cold has any diabetogenic action, equivalent to the removal of a small fraction of a gram of pancreatic tissue, this action should be demonstrable in such tests.

Twenty-five dogs were used for this investigation, with removal of such portions of pancreas as were known to produce a close approach to diabetes.[1] These experiments included fasting and fixed diets, also single and repeated operations, the latter as usual removing successive bits of tissue till diabetes resulted. Comparisons were made between dogs kept in the warmth and others kept in the cold, and also in the same animals by sudden changes from one environment to the other. The animals chosen ranged from small short-haired dogs which were highly sensitive to the cold and might sometimes be unduly depressed by it, to large woolly dogs which scarcely shivered in the winter weather. The general technic of such experiments is sufficiently clear from the preceding papers, so that brevity may be served by omitting protocols. In two instances the change from warmth to cold seemed responsible for a definite but transitory glycosuria. Otherwise the results were negative, and the conclusion was established positively that there is no demonstrable difference in the amount of pancreatic tissue that

[1] All operations were performed under ether anesthesia.

must be removed to produce diabetes in dogs in warm or cold environment. An effect of cold upon the islands of Langerhans was also not observable.

Although cold has no diabetogenic influence whatever in the sense of this test, it thus merely conforms to the rule that the most powerful functional influences avail little in comparison with the smallest fraction of a gram of healthy pancreas tissue. As agencies which are negative in this respect sometimes appreciably influence the course of an existing diabetes, some experiments concerning hyperglycemia and glycosuria were performed upon dogs with various grades of carbohydrate tolerance. For this purpose the animals' cages were transferred from a comfortably warm room to a refrigerator room kept at approximately freezing temperature. The observations are arranged in a series chiefly according to descending assimilative capacity.

Dog C3–36

TIME	PLASMA SUGAR	REMARKS
	per cent	
August 6		
10:00 a.m.....	0.128	At summer temperature
3:40 p.m.....	0.116	Immediately after this bleeding, transferred to ice room
5:50 p.m..	0.098	
August 7		
10:00 a.m.....	0.113	
4:00 p.m.....	0.100	Immediately after this bleeding, transferred from ice room to summer temperature
6:00 p.m.....	0.095	
9:25 p.m.... . .	0.105	

The normal dog C3-36, weighing 17 kilos, was placed in the ice room after the bleeding at 3:40 p.m. on August 6, and left there until after the bleeding at 4 p.m. on August 7. The usual diet of bread and soup was fed each evening after the final blood sample was taken. The dog was in excellent condition and powerfully muscled, but very short haired, and shivered continuously in the cold. The plasma sugar seemed to be affected very slightly if at all. At any rate, no elevation by cold was observed.

Dog B2-00, mentioned several times in previous papers, in August, 1915 was close to the verge of diabetes, but had been free from glycosuria on fixed bread diet for several months. Exercise had proved able to modify the blood sugar and the glucose assimilation considerably,

and an experiment with cold was therefore performed under similar conditions. The diet was fed each evening after the last blood test. There was no glycosuria. The plasma sugar was slightly lower at the low temperature.

TIME	PLASMA SUGAR	REMARKS
	per cent	
August 5		
11:30 a.m.....	0.111	
2:30 p.m.....	0.117	At summer temperature
August 6		
9:30 a.m.....	0.110	
3:30 p.m.... ...	0.098	Immediately after this bleeding, transferred to ice room
5:45 p.m.....	0.085	
August 7		
9:30 a.m.....	0.098	
3:30 p.m.....	0.107	Immediately after this bleeding, transferred from ice room to summer temperature
6:00 p.m.....	0.125	
9:30 p.m.....	0.112	

Dog B2-01

PLASMA SUGAR			TIME
September 21, Cold	September 29, Control	October 6, Exercise	
per cent	*per cent*	*per cent*	
0.116	0.106	0.105	Blood before feeding
0.192	0.256	0.147	1 hour after feeding
0.106	0.147	0.148	3 hours after feeding
0.131	0.103		6 hours after feeding

Dog B2-01, likewise near the verge of diabetes, received 56 grams Merck anhydrous glucose (4 grams per kilo) in 30 per cent solution by stomach tube on three days, respectively in the cold, in warmth at rest, and with treadmill running. The control day showed the highest plasma sugar curve, while both cold and exercise seemed to depress it.

Dog B2–02

PLASMA SUGAR			URINE SUGAR		TIME
July 20, Control	September 23, Cold	October 15, Exercise	July 20, Control	September 23, Cold	
per cent	*per cent*	*per cent*	*per cent*	*per cent*	
0.144	0.118	0.114	0	0	Before feeding
0.270	0.228	0.111	4.50 (13 cc.)	Slight	1 hour after feeding
0.149	0.115	0.100	1.85 (84 cc.)	0	3 hours after feeding
0.112	0.100		0	0	6 hours after feeding

Dog B2–02 was known to have very mild latent diabetes, but was continuously free from glycosuria on bread diet. Tests similar to those of dog B2-01 were carried out with the giving of 30.5 grams Merck glucose (3 grams per kilo) by stomach tube. Cold seemed to reduce hyperglycemia and glycosuria as compared with the control day. On the exercise day there was by far the lowest plasma sugar and no glycosuria.

Dog D4–29

PLASMA SUGAR			TIME
October 4, Control	October 9, Cold	October 10, Control	
per cent	*per cent*	*per cent*	
0.093	0.120	0.147	Blood before feeding
0.307	0.357	0.256	1 hour after feeding
0.307	0.455	0.202	2 hours after feeding
0.322	0.400	0.235	3 hours after feeding
0.307	0.416	0.222	4 hours after feeding
0.313	0.364	0.250	5 hours after feeding
	0.322	0.206	8 hours after feeding
	0.125	0.200	13 hours after feeding

Dog D4–29. Female; mongrel; age 2 years; good condition; weight 11.5 kilos. September 28, 1916, removal of pancreatic tissue weighing 26.9 grams. Remnant about main duct estimated at 3.8 grams ($\frac{1}{8}$). The removal of 0.65 gram additional tissue on October 16 was necessary to produce diabetes. In the interval the tests shown in the table were performed, with the feeding of 200 grams bread and 150 grams glucose on each occasion (together with 200 grams talcum powder as a precaution against diarrhea). On October 9 the dog was transferred to the cold room immediately after feeding. The plasma sugar curve ran noticeably higher on this day than on the control days, but yet was lower at the end of 13 hours, as if more of the available carbohydrate had either been excreted or consumed by shivering. An accident prevented comparison of the glycosuria.

Dog B2–86. The history of this animal was given in paper 2 of the preceding series (3). The animal had been on the verge of diabetes with an unusually large pancreas remnant, and enormous quantities of bread and glucose had been necessary to keep up glycosuria, but by July 21 this was disappearing and the appetite was failing. Therefore the expedient was adopted of raising the animal's

metabolism to the highest level possible, by exercising him on the treadmill to the limit of his strength daily, and keeping him in the ice room all the rest of the time, in the endeavor to bring on diabetes either by stimulation of appetite or by any direct influence upon the pancreas. July 22 was occupied in this manner. July 23, exercise was deferred until a feeding experiment with 400 grams bread and 500 grams glucose could be carried out in the ice room in comparison with the one at summer temperature on July 21.

TIME	PLASMA SUGAR	
	July 21, at summer temperature	July 23, at freezing temperature
	per cent	*per cent*
Before feeding	0.124	0.100
2 hours after	0.147	0.128
7 hours after	0.141	0.141

The lower sugar curve on July 23 may have been partly the result of the preceding day's exercise, but at least augured failure for the undertaking. The program of combined exercise and cold was continued daily, with addition of as much as 600 grams of glucose to the bread diet, up to the time of the second operation on August 7. The strong animal merely thrived on the program, and such diabetic tendency as had seemed to be present disappeared.

Dog B2–71. June 3, 1914, at a normal weight of 14.7 kilos, nine-tenths of the pancreas were removed. In July, 1915 the dog was still in a state of moderate diabetes, kept sugar-free on meat diet, at a weight of 12.5 kilos, and was used for exercise and other experiments. Some tests were then performed with feeding 50 grams of bread, following only the urine without blood analyses. The regular lung diet was given each evening after completion of the test.

TIME	URINE		REMARKS
	Volume	Glucose	
	cc.	*per cent*	
July 28			
2:30 p.m.		0	Fed 50 grams bread
5:00 p.m.	38	0.28	
July 29			
2:30 p.m.		0	Fed 50 grams bread and placed in ice room
5:00 p.m.	47	0	Returned to summer temperature
July 30			
10:00 a.m.		0	Fed 50 grams bread and placed in ice room
5:00 p.m.	214	Trace	Returned to summer temperature
July 31			
10:00 a.m.		0	Fed 50 grams bread
5:00 p.m.	275	0.4	

In this experiment there was distinctly greater glycosuria on July 28 and 31 in warm summer weather than on July 29 and 30 at freezing temperature.

Dog C3–00. Female; fox terrier mongrel; white and brown; age 5 years; good condition; weight 4.5 kilos. May 6, 1915, removal of pancreatic tissue weighing 11 grams. Remnant about main duct estimated at 1.25 grams ($\frac{1}{9}$). As sometimes happens in small dogs, glycosuria was absent on full meat diet even with this small pancreas remnant. On May 15 a change to bread and soup promptly brought heavy glycosuria, which ceased with a return to meat diet on May 21. A fixed diet of 750 grams lung was then given daily, but was not always eaten completely. After continuous absence of glycosuria, the dog was transferred to the ice room on May 29, and in the following 24 hours excreted 0.45 per cent sugar in 375 cc. urine. Glycosuria then continued absent on the same diet as before, until on June 7 the dog was removed from the ice room. At summer temperature a return to bread and soup diet produced immediate heavy glycosuria. Accordingly, in this experiment cold failed to maintain glycosuria on meat diet in a dog which was demonstrably diabetic as proved by glycosuria on bread diet.

In August the same dog was tested with the aid of plasma sugar analyses on a regular diet of 500 grams lung and 50 grams suet. There was no glycosuria.

TIME	PLASMA SUGAR	REMARKS
	per cent	
August 10		
10:45 a.m.....	0.135	Immediately after this bleeding, transferred to ice room
12:00 noon.....	0.122	
4:45 p.m.....	0.141	
August 15		
12:30 p.m.....	0.182	
August 16		
12:45 p.m.....	0.098	
4:45 p.m.....	0.112	Immediately after this bleeding, transferred from ice room to summer temperature
August 18		
12:00 noon.....	0.167	Summer temperature
5:25 p.m.....	0.143	

Dog B2–88

PLASMA SUGAR					TIME
November 15, Control	November 18, Control	November 20, Cold	November 23, Cold	November 26, Exercise	
per cent	*per cent*	*per cent*	*per cent*	*per cent*	
0.128	0.189	0.131	0.122	0.162	Blood before feeding
	0.250	0.222		0.263	½ hour after feeding
	0.257	0.233		0.189	2 hours after feeding
0.286			0.271		4 hours after feeding

Dog B2–88, with mild diabetes, received test meals of 200 grams bread and 100 grams beef lung. The plasma sugar curve seemed to vary chiefly according to the initial figure, without much influence of any of the special measures employed. Thus, both figures on November 15 are a trifle higher than on November 23 in the refrigerator. Also the differences between November 18, 20 and 26 seemed to be governed chiefly by the level on which the plasma sugar started. Cold at least did not elevate the plasma sugar.

Dog B2–58. Female; mongrel; yellow; age 3 years; good condition; weight 11.8 kilos. Fasting was begun May 4, 1914, and by May 21 the weight was reduced to 9.1 kilos. It was thus possible to remove $\frac{1}{1}\frac{1}{2}$ of the pancreas on this date with only a moderate degree of diabetes resulting. After various tests, the tolerance was spared by a low protein diet, which could be gradually increased by October 20 to 1 kilo of beef lung daily, without glycosuria. The weight gradually rose by December 24 to 12.3 kilos, on which date the first glycosuria appeared. About the same time the dog began to leave part of the diet uneaten, and the weight thus fluctuated and in general fell. The partial checking of glycosuria, and its daily variations, are thus accounted for. Beginning January 6, 1915, the dog's cage was kept outdoors, in order to test the effect of cold upon the diabetes either directly, through stimulation of appetite or in any other way. The dog had time to become acclimated because the weather was fairly mild at first, but after January 20 it turned decidedly colder, and there were several days with considerable snow and ice. As the dog was short-haired, she was shivering practically continuously while outdoors, but passed through even the coldest weather without actual impairment of health. The rectal temperature remained normal. Contrary to expectation, the amount of food eaten was not appreciably different in the cold environment. After February 12 the glycosuria was checked by fasting. The experiment seems to indicate a slight increase of glycosuria by cold, but the influence certainly was not great.

Dog B2–58

DATE	WEIGHT	URINE	
		Volume	Glucose
	kgm.	*cc.*	*per cent*
December 24		320	1.0
25		585	0.5
26	12.8	470	Faint
27		385	0.2
28		950	Faint
29		355	0
30	12.7	384	0
January 1		360	0
2		500	0
3		632	0
4		650	Faint

Dog B2–58—Concluded

DATE		WEIGHT	URINE	
			Volume	Glucose
		kgm.	*cc.*	*per cent*
January	5		480	0
	6	12.7	330	0
	7	12.4	502	0
		Today placed	in cage outdoors	
	8		431	0
	9		307	0
	10		575	0
	11	11.1	713	0
	12		766	0
	13	11.2	646	0
	14		954	0
	15	10.9	698	0
	16		382	0
	17	Not	collected	
	18	11.1	1849	0
	19		988	0 6
	20	11.3	995	0
	21		965	0.7
	22	11.1	781	0.9
	23	11.2	609	0.5
	24		740	0.9
	25	10.9	700	Faint
	26	11.1	749	0
	27	11 0	900	V. Faint
	28	11 3	731	1.5
	29	11.5	630	1.0
	30	11.5	156	2.0
	31		820	1.1
February	1	11 3	1036	1.0
	2	11 3	1300	0.8
	3	11.0	950	2.0
	4	11 1	785	1.8
	5	11.4	1157	2.0
	6	11.8	605	2.5
		Today moved into	animal room	
	7		650	1.0
	8	11 5	973	0.9
	9	11.5	649	0.4
	10	11.7	733	0.7
	11	11.5	815	Faint
	12		600	Faint

Dog B2–89. This female mongrel, weighing 13 kilos, underwent partial pancreatectomy on April 12, 1915, leaving a remnant of $\frac{1}{11}$. Under regulated diets, the condition by the end of June was such that glycosuria was absent at a weight of 10.3 kilos on a diet of 1 kilo of lung, but substitution of 250 grams lung by 50 grams bread (making 750 grams lung and 50 grams bread) caused glycosuria of 0.75 per cent in 462 cc. urine on June 30 and 0.71 per cent in 542 cc. urine on July 1. On the latter date the diet of 1 kilo of lung without bread was resumed, and glycosuria immediately ceased (4). On July 2 the dog was transferred to the ice room, in order to test whether the influence of cold would amount to as much as the above difference between carbohydrate and protein. The dog was left in the ice room until July 26. Traces of glycosuria were present on most days during this time, but only twice reached titratable amounts (0.33 per cent on July 14, 0.25 per cent on July 21). A single day of exercise on July 22 abolished the glycosuria, which returned on July 24. It was present also on July 25 and 26, and then was continuously absent during a control period up to August 5 at summer temperature. As the weight fell to 9.7 kilos during the period in the refrigerator, the experiment seems to indicate a slight increase of diabetic tendency due to cold. The difference due to temperature, however, was evidently less than the difference between the preformed carbohydrate of 50 grams of bread and its approximate equivalent of potential carbohydrate in protein. The tolerance had fallen somewhat, for the giving of 50 grams of bread on August 15 resulted in a glycosuria of 1.4 per cent.

Glycosuria remained absent on the lung diet to September 17. On that day at 9:30 a.m. the plasma sugar was 0.143 per cent, the rectal temperature 101.4° F. The dog was then placed in the ice room fasting. At 4 p.m. the plasma sugar was 0.151 per cent, the rectal temperature 100.7°F. The usual lung diet was then fed and the dog left in the ice room. The next morning there was a glycosuria of 0.42 per cent in 567 cc. urine, and the plasma sugar at 9:30 a.m. was 0.164 per cent. The urine of the next 24 hours was 492 cc., with 1.9 per cent sugar. At 9:30 a.m. on September 19 the rectal temperature was 101.5°F. Glycosuria then ceased abruptly, as though the reserve of extra carbohydrate had been exhausted. At 9:30 a.m. on September 20 the plasma sugar was 0.146 per cent. The dog was then transferred to the warm animal room, and at 9:30 a.m. on September 21 the plasma sugar was 0.133 per cent, the rectal temperature 101.7°F.

During the following days the weather turned colder. The night of September 23–24 was particularly sharp, and the door of the animal room blew open, so that the room was cold and the dogs all shivering. This dog was one of five potentially diabetic animals (out of about twenty) which had been kept sugar-free on regulated diets and which showed sudden glycosuria on this night.

Subsequently, at a body weight of 15 kilos and correspondingly reduced tolerance, a comparison was made in this dog of the feeding of 1 kilo of meat in a warm room and in the ice room.

TIME	PLASMA SUGAR	
	November 26, (warm room)	December 3, (ice room)
	per cent	*per cent*
Before feeding	0.200	0.208
2 hours after feeding	0.218	0.278
5 hours after feeding	0.208	0.313
Glycosuria for the period	0	4.08 grams

The later observations showed a decided influence of cold for the production of hyperglycemia and glycosuria. It is possible that this effect became greater as the diabetes became more severe.

Dog B2–79

PLASMA SUGAR		URINE SUGAR		TIME
November 23, Warm	November 30, Cold	November 23, Warm	November 30, Cold	
per cent	*per cent*	*per cent*	*per cent*	
0.133	0.250	0	Faint	Blood before feeding
0.213	0.416	Faint	5.00	1 hour after feeding
0.159	0.525	Faint	5.90	4 hours after feeding
0.189	0.435	0	3.53	6 hours after feeding

Dog B2–79 was an animal which had long been kept in a stage of diabetes such that there was marked hyperglycemia and occasional glycosuria on a diet of 1 kilo of beef lung, the condition being held in check by fasting when necessary. The above record shows a strong contrast in hyperglycemia and glycosuria on days spent in a warm room and in the ice room respectively. The rise of blood sugar after eating the usual kilo of lung was somewhat similar on the two days, but the entire curve was on a much higher level in the cold environment. It was not established that this difference was due entirely to the temperature, because no comparison was made of the blood sugar before and after moving into the refrigerator on November 30. Therefore on December 2 the plasma sugar was determined fasting at 11:40 a.m. and found to be 0.164 per cent. The dog was then moved into the cold room, and at 5:40 p.m. the plasma sugar (still fasting) was found to be 0.185 per cent.

Likewise dog C3–19, weighing 13.8 kilos, was partially depancreatized on June 23, 1915, leaving a remnant of $\frac{1}{11}$ to $\frac{1}{12}$. In August at a weight of 11 kilos, the degree of diabetes was such that a diet of 500 grams lung was slightly in excess of the tolerance. On August 7 the fasting plasma sugar at 10:30 a.m. was 0.156 per cent and at 4:30 p.m. 0.120 per cent. The dog, still fasting, was then moved to the ice room, where the plasma sugar was found to be 0.167 per cent at 7 p.m. and 0.133 per cent at 9:45 p.m. Here a falling blood sugar due to fasting was evidently raised by cold, and then continued to fall slightly.

DISCUSSION

The elevation of blood sugar by cold is so generally accepted as a truism that it was a surprise to encounter instances in which the sugar was little changed or actually lower in a cold environment. It was not feasible to extend the investigation further into the physiological reaction to cold. Supposedly the blood sugar is governed by two factors, namely the mobilization and the disposal of carbohydrate. It is conceivable that in a perfectly smoothly working reaction the two may exactly balance. Under some conditions shivering may perhaps reduce the blood sugar like other forms of muscular activity. Under other conditions hyperglycemia may occur, particularly when the cold stimulus is sufficiently violent, as for example in the case of plunging into ice water. Here the stimulation is so powerful, sometimes to a pathological degree, that a correspondingly excessive sugar discharge may be expected, and in the most extreme cases possibly the utilization of sugar suffers somewhat. Hyperglycemia and glycosuria may be more readily produced when the utilization of sugar is specifically impaired, as in the more severe grades of diabetes. In a similar way exercise sometimes raises the blood sugar instead of lowering it.

Violent or pathological stimulation by cold was not applicable in experiments designed to produce diabetes, because the animals' health would suffer and ill health would be the surest way to spoil the result and prevent diabetes. A more powerful temporary discharge of sugar might occur, but it would cease as soon as the immediate store was exhausted. Such discharge and cessation was actually seen in certain of these experiments, without any lasting diabetogenic effect. As usual, clear thinking requires a distinction between diabetes, which is deficiency of the pancreatic function, and mere glycosuria. The mere excessive discharge of sugar from the glycogen depots is not diabetes, for the power of utilization may remain unimpaired. This has been abundantly proved, for example, in such a condition as epinephrin glycosuria (5). It is also not diabetes if the utilization of sugar is depressed by any extraneous mechanism, such as the chilling of the muscles or their nervous supply, but only if the impairment of utilization is due to impairment of the pancreatic function. If the glycosuria produced by cold is regarded as a diabetes, cold must be a very powerful diabetogenic agent to cause even a temporary diabetes in a normal animal, for it must thus temporarily paralyze about nine-tenths of the function of the dog's pancreas. Trial of this agent in animals

depancreatized almost to the point of diabetes proves that it possesses no such power; and as the effect in these animals is not greatly different from that in normal animals, cold evidently does not act by direct depression of the pancreatic function. Its indirect influence upon diabetes through increasing metabolism is a much more delicate point to demonstrate, and the question is answered only somewhat doubtfully in the affirmative by some of the above experiments.

CONCLUSIONS

1. Cold environment, such as did not lower the rectal temperature to any important extent, in some instances failed to affect the plasma sugar of dogs or slightly lowered it, but in the majority of experiments produced hyperglycemia and sometimes glycosuria. These were produced more easily and in higher degree in proportion as the power of sugar utilization was impaired, i.e., as the diabetes was more severe.

2. The power to produce glycosuria is to be distinguished from the power to produce diabetes. There is no demonstrable difference in the proportion of pancreatic tissue that must be removed to produce diabetes in dogs in warm or cold environment, and it was proved by successive operations upon the same animals that the influence of cold is not equivalent to the removal of the smallest fraction of a gram of pancreatic tissue. In animals already diabetic, the course of the diabetes in a few instances seemed to be influenced slightly for the worse, so as perhaps to warrant the conclusion that cold imposes an increased burden upon the pancreatic function by increasing metabolism. But the slightness of this influence is emphasized by control experiments; for example, it amounts to less than the difference between the preformed carbohydrate of 50 grams of bread and the approximate equivalent of potential carbohydrate in protein.

3. The impression that diabetic patients do worse in cold weather is probably explainable by the discomfort of chilliness when they are undernourished, the tendency to take more food, and sometimes by the limitation of exercise. These may be important sometimes from a practical standpoint, but any direct influence of climate upon diabetes must be very slight if it exists.

BIBLIOGRAPHY

(1) ALLEN: Studies concerning glycosuria and diabetes, 1913, 562.
(2) ALLEN, STILLMAN AND FITZ: Rockefeller Inst. Monograph no. 11, 1919, 471.
(3) ALLEN: Journ. Exper. Med., 1920, xxxi, 385.
(4) For the greater glycosuric effect of preformed carbohydrate as compared with protein in mild diabetes. See Journ. Exper. Med., 1920, xxxi, 555 and 397 to 399.
(5) LUSK: Proc. Soc. Exper. Biol. and Med., 1913–14, xi, 49. Cf. also paper 12 of this series.

EXPERIMENTAL STUDIES IN DIABETES

Series II. The Internal Pancreatic Function in Relation to Body Mass and Metabolism

8. The Influence of Extremes of Age upon the Production of Diabetes

FREDERICK M. ALLEN

From the Hospital of The Rockefeller Institute for Medical Research, New York

Received for publication August 10, 1920

An influence upon the production of diabetes may conceivably be expected from the conditions of extreme age or youth.

Senility. The relation of senility to experimental diabetes may have interest from at least three standpoints: *a*, The total metabolism is known to be slightly lowered in old age; *b*, diabetes in elderly patients is generally characterized by a mild and prolonged course; *c*, the increasing incidence of diabetes with the advance of age suggests the possibility of functional or organic impairment of the pancreas.

Toothless decrepit senility is familiar in dogs, and there is reason to expect as great metabolic changes as in aged human beings. Obesity is also common in such animals, so that a number of them had to be included in a previous paper (1). A comparison of the relation of pancreas weight to body weight was made in fourteen dogs, which showed extreme senility together with an average nutritive state.

From a comparison of table 1 with a similar study of normal adult dogs (2), it may be inferred that there is no gross change, in particular no atrophy of the pancreas accompanying senility.

The susceptibility of obese senile dogs to diabetes could seldom be determined, owing to the sudden death to which they are subject following pancreas operations, as previously mentioned (3). Such operations were performed without accidents in senile dogs without obesity, and these were used for diabetic experiments in the same way as younger dogs. No experiments were performed to determine the precise proportion of pancreas that must be removed to produce diabetes, but the incidental observations were sufficient to exclude any marked differences from average adult dogs. The senile animals were some-

what weaker and more subject to loss of appetite and cachexia. Otherwise their diabetes ran a course indistinguishable from that of younger animals. The experimental answers may therefore be stated in the following form, corresponding to the questions raised above.

TABLE 1

Relation of pancreas weight to body weight in senile dogs

NUMBER	BODY WEIGHT	PANCREAS WEIGHT	PANCREAS PER KILOGRAM BODY WEIGHT
	kgm.	*grams*	*grams*
1	4.0	15.0	3.75
2	4.4	7.0	1.59
3	5.8	9.5	1.64
4	8.8	23.1	2.62
5	11.3	19.6	1.73
6	11.3	24.4	2.16
7	11.5	25.6	2.23
8	11.7	32.1	2.74
9	14.2	53.6	3.78
10	15.0	33.6	2.24
11	16.0	20.2	1.26
12	21.3	46.4	2.18
13	21.8	39.6	1.81
14	25.1	41.4	1.65

a. The lowered metabolism characteristic of senility does not render dogs less susceptible to diabetes from pancreatic resection. As the reduction of metabolism with age is slight, and the method of judging susceptibility by the size of the pancreas remnant with which diabetes occurs is a crude one according to former observations, too much stress need not be laid upon these negative findings.

b. A more decisive observation is that diabetes does not run any slower course in senile animals, but follows the same rapid progress which is generally characteristic of diabetes in dogs.

c. If there were any anatomic or functional deterioration of the pancreas with age, a remnant of a given size might be expected to be less efficient in preventing diabetes than in younger animals. An increased susceptibility to diabetes in this sense is excluded by the observations. The microscopic study, as reported in subsequent papers, showed no visible abnormalities resulting from simple senility. Pancreatitis is much rarer in dogs than in human beings, and is apparently due to causes independent of age. Also there is no such arteriosclerosis

in senile dogs as in man, and it can by no means be said that a dog "is as old as his arteries." Also, as previously mentioned (2), the pancreas remnant of a senile dog may possess as great power of hypertrophy as that of any younger animal.

Numerous glucose tolerance tests have been performed indiscriminately upon old and young adult dogs, and no indications of alteration of assimilation with age have been found.

In general these observations, made upon a species practically free from the pancreatic changes to which man is subject with advancing years, indicate that the rising incidence and special characteristics of diabetes in older persons are due to the changes in question and not to senility *per se*.

Youth. The outstanding feature of experimental interest is the characteristically rapid and fatal course of diabetes in children. This has often been attributed hypothetically to their high metabolism, which imposes a heavier burden upon the pancreatic function. There is also good evidence that this rapid downward progress indicates a susceptibility of the islands of Langerhans to rapid destruction by hydropic degeneration. As there are occasional cases of acute and severe diabetes in the aged and of mild and prolonged diabetes in children, there is no absolute distinction on the basis of either the level of metabolism or the island changes.

Diabetes is also less frequent in children than in older persons. In seeking possible reasons, it might be imagined that the youthful pancreas is larger in proportion to the body, that it is anatomically richer in islands or that these have stronger functional power, or that the capacity for regeneration after injuries is greater. According to work previously reviewed (4), especially that of Bensley, the pancreas at birth probably contains as many islands as the adult organ, but during early life there is probably a loss followed by a gradual new formation of islands. Observations on the gross relations of the pancreas in puppies are contained in tables 2 to 6. They indicate, in comparison with those on adult dogs (2), that the ratio of pancreas weight to body weight in puppies is not large but rather small in proportion to what might be expected from the small size of the animals; that the tendency to regeneration is often marked but yet not in excess of that often found in adults; and that the tendency to diabetes is at least no greater and often is distinctly less than in adult dogs.

In qualification of this statement, it should be noticed that the observations do not exclude possible alterations of the ratio of pancreas

TABLE 2

Litter of black and tan mongrel pups. Weight of mother, 17 kilos

NUMBER	SEX	AGE	BODY WEIGHT	PANCREAS WEIGHT	PANCREAS WEIGHT PER KILOGRAM
			kgm.	*gram*	*grams*
1	Male	Newborn	0.3	0.6	2.0
2	Male	Newborn	0.3	0.6	2.0
3	Female	Newborn	0.2	0.4	2.0
4	Female	Newborn	0.3	0.5	1.67
5	Male	Newborn	0.3	0.6	2.0
6	Female	Newborn	0.3	0.7	2.33
7	Male	Newborn	0.4	0.8	2.0
8	Female	Newborn	0.3	0.9	3.0
9	Male	Newborn	0.3	0.6	2.0
10	Male	Newborn	0.3	0.8	2.66
11	Female	Newborn	0.3	0.6	2.0

TABLE 3

Litter of harrier mongrel pups. Weight of mother 13 kilos

NUMBER	SEX	AGE	BODY WEIGHT	PANCREAS WEIGHT	PANCREAS WEIGHT PER KILOGRAM
			kgm.	*gram*	*grams*
1	Female	1 day	0.2	0.3	1.5
2	Male	1 day	0.2	0.3	1.5
3	Male	1 day	0.3	0.6	2.0
4	Male	1 day	0.3	0.4	1.33
5	Male	1 day	0.3	0.7	2.33
6	Male	1 day	0.2	0.7	3.50

TABLE 4

Litter of spaniel mongrel pups. Weight of mother, 16 kilos

NUMBER	AGE	BODY WEIGHT	PANCREAS WEIGHT	PANCREAS WEIGHT PER KILOGRAM
		kgm.	*grams*	*grams*
1	Slightly premature	0.2	0.7	3.5
2	Slightly premature	0.2	0.6	3.0
3	Slightly premature	0.3	0.8	2.67
4	Slightly premature	0.3	0.6	2.0
5	2 days	0.3	0.9	3.0
6	3 weeks	0.6	2.1	3.5

weight to body weight with age. In other words, there is no proof that a puppy having a certain ratio will maintain this same ratio up to adult age. In tables 4 and 5 no great change was noticed in the

TABLE 5

Litter of yellow and brown mongrel pups. Weight of mother, 21.5 kilos

NUMBER	AGE	BODY WEIGHT	PANCREAS WEIGHT	PANCREAS WEIGHT PER KILOGRAM
		kgm.	*grams*	*grams.*
1	½ week	0.3	1.1	3.67
2	½ week	0.4	1.7	4.25
3	½ week	0.4	1.3	3.25
4	2 weeks	0.6	2.6	4.33
5	2 weeks	0.7	2.8	4.00

TABLE 6

Relation of pancreas weight to body weight in puppies

NUMBER	AGE	BODY WEIGHT	PANCREAS WEIGHT	PANCREAS WEIGHT PER KILOGRAM
		kgm.	*grams*	*grams*
1	1 week premature	0.1	0.2	2.0
2	1 week premature	0.1	0.2	2.0
3	1 month	0.7	2.2	3.14
4	1 month	1.5	7.1	4.74
5	1½ months	2.1	4.1	1.95
6	1½ months	1.3	2.6	2.00
7	2 months	0.9	3.5	3.89
8	2 months	2.3	5.1	2.22
9	2 months	2.0	5.9	2.95
10	2 months	1.8	5.8	3.22
11	2 months	4.3	8.4	1.95
12	2 months	2.3	7.4	3.21
13	2½ months	1.8	7.4	4.10
14	2½ months	2.5	5.7	2.28
15	3 months	2.5	12.2	4.89
16	3 months	2.3	5.2	2.26
17	5 months	3.9	13.8	3.54
18	7 months	2.5	11.4	4.55

ratio up to 2 or 3 weeks of age, using other pups of the same litter as controls. The ratios varied widely among the different pups in table 6. They were sometimes larger in the smaller breeds, as found for adult dogs (2), but the rule was not uniform. The state of nutrition

TABLE 7

Partial pancreatectomies in puppies

NUMBER	AGE	BODY WEIGHT	PANCREAS WEIGHT	PANCREAS WEIGHT PER KILOGRAM	WEIGHT OF REMNANT	SIZE OF FRACTION	HYPERTROPHY	REMARKS
	months	*kgm.*	*grams*	*grams*	*grams*		*grams*	
1	1	3.0	13.2	4.4	0.8	$\frac{1}{16}$–$\frac{1}{17}$	0.8–1.02	
2	2	1.9	9.7	5.1	1.3	$\frac{1}{4}$–$\frac{1}{5}$	1.3–5.7	Transitory glycosuria. Mild diabetes produced by removal of additional 1.2 grams
3	2	1.7	8.9	5.2	0.7	$\frac{1}{13}$–$\frac{1}{14}$	0.7–0.4	Cachexia. Weight 1 kgm. at autopsy. Probably mild diabetes
4	3	2.5	11.0	4.4	0.5	$\frac{1}{22}$	0.5–0.6	Cachexia. Mild diabetes
5	3	2.1	10.8	5.1	3.0	$\frac{1}{3}$–$\frac{1}{4}$		Diabetes not produced even by circulatory stasis
6	3	3.9	17.8	4.6	5.7	$\frac{1}{3}$		Diabetes not produced even by circulatory stasis
7	3	2.0	16.0	8.0	2.7	$\frac{1}{6}$	2.7–5.3	No diabetes
8	3	2.1	8.3	3.9	0.8	$\frac{1}{10}$		Thin, no glycosuria
9	4	3.7	15.8	4.3	1.5	$\frac{1}{10}$		Assimilated 5 grams of glucose per kilogram
10	4	8.4	27.6	3.3	4.6	$\frac{1}{5}$		Removal of 3.6 grams additional tissue in 6 operations required to bring on diabetes. Remnant at autopsy was then 1.1 grams
11	5	1.9	7.8	4.1	0.6	$\frac{1}{3}$	0.6–1.0	
12	5½	5.3	25.8	4.9	4.4	$\frac{1}{6}$	5.4–8.4	No diabetes
13	6	8.0	22.5	2.8	9.3	$\frac{1}{2}$–$\frac{1}{3}$		
14	6	3.8	8.5	2.2	1.0	$\frac{1}{3}$–$\frac{1}{5}$		
15	7	5.9	20.1	3.4	1.7	$\frac{1}{12}$		Peritonitis, no glycosuria
16	7	5.9	15.0	2.54	1.6	$\frac{1}{9}$–$\frac{1}{10}$		Diabetes stopped by distemper
17	7	3.4	9.3	2.74	1.5	$\frac{1}{6}$		Cachexia, no glycosuria
18	7	4.4	8.9	2.02	1.0	$\frac{1}{10}$		Diabetes, mild, transitory
19	8	7.2	19.8	2.75	1.8	$\frac{1}{11}$		

TABLE 7—*Concluded*

NUMBER	AGE	BODY WEIGHT	PANCREAS WEIGHT	PANCREAS WEIGHT PER KILOGRAM	WEIGHT OF REMNANT	SIZE OF FRACTION	HYPERTROPHY	REMARKS
	months	*kgm.*	*grams*	*grams*	*grams*		*grams*	
20	9	5.8	21.5	3.71	1.3	$\frac{1}{17}$		Diabetes prevented by preliminary fast
21	9	2.7	10.5	3.90	1.0	$\frac{1}{10}$		Peritonitis, no diabetes
22	9	7.4	24.8	3.36	2.5	$\frac{1}{10}$		Severe diabetes even during fasting
23	9	7.1	26.2	3.69	3.0	$\frac{1}{2}$	3.0–1.95	Severe diabetes checked by cachexia. Emaciated to 5.3 kgm.
24	9	10.3	30.9	3.00	3.1	$\frac{1}{10}$	Considerable	
25	11	4.1	14.3	3.50	1.1	$\frac{1}{3}$	1.1–2.2	Cachexia, no diabetes

or rate of growth is probably an important factor. The differences found among animals of the same litter at the same age in tables 2 and 3 interfere seriously with studies based on any method of controls.

Partially depancreatized puppies are especially liable to cachexia from respiratory infections or diarrhea, so that diabetes is often thus suppressed and the experiments spoiled. The above statement concerning the relatively slight disposition to diabetes is based on animals which remained vigorous and thriving after operation. It is the more striking in view of the fact that the sugar tolerance of the young is distinctly less than that of adult normal animals (5). A series of observations was made upon the question whether partial pancreatectomy short of diabetes interferes with the growth or health of puppies, with negative results except for the temporary backset due to the operation, and a possible specific inhibition of development of sexual and other adult characters. Anything in the nature of a masked diabetes or other fatal metabolic deficiency seemed to be excluded.

As explained in a subsequent publication on acidosis, the expectation that puppies might prove more susceptible than adult dogs to diabetic acidosis was entirely disappointed. Acidosis was absent throughout the reported experiments except where specially mentioned.

As puppies approach adult age, they approach more closely to the adult behavior respecting diabetes. After about the ninth month they generally react like adults except for susceptibility to distemper. Nevertheless in pup 18 in table 7 it is noticeable that diabetes was

transitory with a pancreas remnant of only $\frac{1}{16}$. Some typical records of younger puppies are the following.[1]

No. G7–49. Male; mongrel; black and white; age 1 month; well nourished; weight 3 kilos. June 25, 1918, removal of pancreatic tissue weighing 12.4 grams. Remnant about main duct estimated at 0.8 gram ($\frac{1}{16}$ to $\frac{1}{17}$). Following operation there was diarrhea and loss of weight but no glycosuria, though the pup lived on bread and milk. July 4, 0.32 gram additional pancreatic tissue was removed. The condition was still cachexia without glycosuria, up to death on July 11. The pancreas remnant weighed 0.7 gram, and was too badly autolyzed for microscopic study. In the tissue removed on July 4, the acini were normal and well filled; islands were present in normal number and size but showed slight distinct vacuolation. This hydropic change makes it probable that hyperglycemia was present, and possibly only the animal's weakness prevented a frank diabetes. Acidosis was absent as usual.

No. D4–35. Female; mongrel; age 3 months; brown and white; well nourished; weight 2.5 kilos. November 9, 1916, removal of pancreatic tissue weighing 10.5 grams. Remnant about main duct estimated at 0.5 gram ($\frac{1}{22}$). Glycosuria was brought on by the feeding of 20 grams meat and 20 cc. milk, but ceased readily with fasting. Death occurred from weakness, notwithstanding a low meat diet, on November 20. The pancreas remnant weighed 0.6 gram, and was not examined microscopically. The pup obviously had a potentially severe diabetes, but differed from an adult dog in that glycosuria did not begin spontaneously and was promptly checked by fasting and low diet, with such a very small pancreas remnant.

No. D4–34 similarly underwent removal of 8.2 grams pancreatic tissue, leaving a remnant estimated at 0.65 gram ($\frac{1}{13}$ to $\frac{1}{14}$). Glycosuria could readily be checked by fasting, but on full meat diet was as high as 1.2 per cent. Notwithstanding the liveliness following operation and the meat diet, the weight and strength failed rapidly. The animal was killed when moribund on the tenth day after operation, when the plasma sugar was 0.05 per cent. The rapid cachexia was the chief point of difference from an adult dog.

No. G7–25. Female; Boston terrier; brindle; age 6 weeks; good nutrition; weight 3.8 kilos. June 17, 1918, removal of pancreatic tissue weighing 13.1 grams. Remnant about main duct estimated at 1.1 gram ($\frac{1}{13}$). Glycosuria remained absent, first on beef lung, then on bread and soup diet, then with addition of 50 grams glucose. June 25, at a weight of 3.5 kilos, 1 gram additional pancreatic tissue was removed, the remnant having undergone marked hypertrophy. Glycosuria remained absent on bread and milk diet, though weight was lost. July 4, 0.95 gram additional pancreatic tissue was removed. The animal was lively and ate considerable lung on July 5, then gradually lost appetite and weight while retaining spirits up to death on July 9. Glycosuria remained absent. The plasma sugar on June 17 was 0.143 per cent, on July 8, 0.162 per cent. The pancreas remnant weighed 0.8 gram. In the various specimens of pancreas from June 17 and July 4 and 9, inflammation and fibrosis were limited to the peripheral areas. In the central portions the acini were well filled, sometimes large and

[1] All operations were performed under ether anesthesia.

irregular. Islands were normal in number and free from vacuolation. Here the persistent absence of diabetes is perhaps attributable to the hypertrophy of the pancreas remnant.

No. E5–40. Male; mongrel, part Newfoundland; age 3½ months; good nutrition; weight 8.4 kilos. May 14, 1917, removal of pancreatic tissue weighing 23 grams. Remnant about main duct estimated at 4.6 grams (⅕). Glycosuria was absent on bread and soup diet, and only transitory with addition of 100 grams glucose.

May 24, additional pancreatic tissue weighing 0.9 gram was removed. There was obvious hypertrophy of the remnant in all three dimensions. Thereafter glycosuria was absent on bread diet, and could be maintained only by increasing additions of glucose, first 50, then 100, then 200 grams daily. The dosage of 200 grams was continued from June 6 to 27 with only traces of glycosuria and with a gain of weight to 9.3 kilos.

June 27, 0.6 gram additional tissue was removed from the pancreas remnant, which again was obviously hypertrophic. Thereafter heavy but transitory glycosuria resulted first from 100 and then from 200 grams glucose added to the bread diet.

July 12, an additional 0.35 gram of pancreatic tissue was removed, the weight then being 9.8 kilos. Glycosuria was then heavy with addition of 200 grams glucose to the bread diet, but ceased July 19.

July 20, an additional 0.4 gram of pancreatic tissue was removed. Glycosuria was then heavy on bread and soup diet, next with addition of glucose up to 200 grams, but was absent after July 29.

August 31, at a weight of 10.6 kilos, glycosuria being still absent on the bread diet with 200 grams glucose, an additional 0.52 gram of pancreatic tissue was removed. Glycosuria was again transitory.

September 7, an additional 0.3 gram of pancreatic tissue was removed with a similar result, glycosuria ceasing September 18.

September 28, an additional 0.55 gram of pancreatic tissue was removed. Glycosuria was then continuous, first with glucose, then on plain bread and soup, till stopped by a change to meat diet on October 22. It may be noted that the downward progress was slow rather than rapid, for the glycosuria of many adult dogs after 3 weeks reaches a point where it cannot be stopped by fasting.

The pup did not thrive on carbohydrate-free diet, suffered from indigestion, diarrhea and loss of weight, and died in cachexia November 16 at a weight of 7 kilos. The blood sugar was low during this period, and there was the usual absence of acidosis.

The pancreas remnant weighed 1.1 gram. Nothing significant was found in the gross autopsy, or in the microscopic examination of the liver, kidneys, adrenals, thyroid and parathyroids. Sections from the pancreas at all the operations and at autopsy showed normal parenchyma and absence of hydropic degeneration. Though the hypertrophy may have gone far toward preventing diabetes, it is evident from the repeated operations and the prolonged carbohydrate excess that this puppy was difficult rather than easy to make diabetic.

No. D4–21. Male; mongrel; age 7 months; good nutrition; weight 6.7 kilos. September 15, 1916, removal of pancreatic tissue weighing 24 grams. Remnant about main duct estimated at 2.9 grams (⅛). Glycosuria at first was absent on

moderate quantities of milk, but was heavy on bread feeding September 18. It was then checked by fasting, and kept absent on a diet of lung, suet and 100 grams bread. The animal thrived and maintained a weight of about 7 kilos.

December 5, a full bread diet was resumed, and heavy glycosuria returned promptly. After 4 days this was stopped as before. The animal grew to adult life on a diet of 400 grams lung, 100 grams suet, and part of the time 100 grams bread or 100 cc. milk. Yeast was added part of the time, with the idea that it and the milk might supply needed vitamines; also an admixture of bonemeal assured adequate salts. The animal became plump and strong at a weight of 8.7 kilos, but remained always undeveloped in mentality, in the size of the sexual organs and the absence of any apparent sexual function; urination was performed squatting, and the puppy contour of the body and puppy-like behavior toward other dogs were retained. Pratt (6) has made similar observations in young dogs with pancreatic atrophy.

November 16, 1917, it was found that the addition of 100 grams bread to the diet produced a glycosuria of 1.3 per cent in 820 cc. of urine. The tolerance was thus evidently lower than before, and thereafter the diet was kept carbohydrate-free (400 to 600 grams of lung and 100 grams of suet). April 6, 1918, the fasting plasma sugar was found to be 0.250 per cent, indicating that hyperglycemia was now continuous. The weight at this time was 9.15 kilos. Traces of glycosuria began in July, at first intermittently, but by July 30 they were continuous and increasing, so that fasting had to be used. The weight at this time had reached its maximum of 9.5 kilos. The animal appeared not obese but in excellent nutrition.

The diet was then gradually built up to 200 grams lung and suet ad libitum, without glycosuria. This diet continued while the writer was in military service. At a visit on September 16, 1918, the animal was found in excellent strength and spirits, weighing 8.3 kilos, with intense glycosuria and hyperglycemia and heavy nitroprusside reactions in urine and plasma. Notwithstanding the fat-rich diet and acidosis, there was no visible lipemia. The condition was ideal for the development of coma, but under the circumstances there was nothing to do but to kill the animal for autopsy.

The body appeared in excellent condition and retained much fat. Except for a very fatty liver and the puppy-like characters noted above, the gross autopsy was negative. The pancreas remnant weighed 3.1 grams. The practical absence of hypertrophy may be noticed.

Microscopically, the liver was crammed with fat even to the periphery of the lobules. Stains with Best's carmine showed absolutely no glycogen except in occasional leukocytes, which stood out prominently in the capillaries by reason of their stuffing of red granules.

The kidneys were normal except for maximal Armanni vacuolation, as seen in routine Zenker specimens stained with methylene blue and eosin. Best's carmine applied to the alcohol fixed specimens showed only a sparse sprinkling of glycogen, so that the vacuoles evidently represented chiefly fat deposit, as demonstrated by fat stains in some other animals but not in this one.

The adrenals showed no more than the average lipoid vacuolation in the cortex, and were normal in other respects.

The thyroid and parathyroids were normal, with average colloid content in the former.

The pancreas remnant was normal in structure and fullness of acini and number and size of islands, but a minority of island cells were maximally swollen and vacuolated.

This puppy, in respect to "spontaneous" downward progress and other features, behaved practically like an adult animal, and most of the history actually represented an adult period of life. Many long experiments of this character were begun for the purpose of testing whether a partial pancreatectomy which did not suffice to produce immediate diabetes in a puppy might result in diabetes after a certain stage of growth had been reached. In other words, the problem was whether a damaged pancreas might lag behind in development so as finally to become inadequate for the demands of a growing body. The best observations would have been those upon much younger animals, but all these failed for one cause or another. It is evident that in many cases the hypertrophy of the pancreas remnant fully compensates for any bodily growth. It is also very doubtful whether an animal which is actually non-diabetic will become diabetic by simple growth. But when the injury results in such a mild diabetes that there are no symptoms on whatever dietary régime is followed, it is entirely possible that diabetes may develop openly at a much later period under the strain of growth and gain of weight. Such a result is exemplified in this experiment, which covers a rather long period in proportion to a dog's life. Such experimental evidence makes it easily comprehensible that diabetic symptoms may follow either soon or late after an injury of the human pancreas; in particular, that glycosuria may be present immediately after an infection, or may be delayed to a time when there is no plain clinical connection with a long antecedent infection which may have been the real cause.

CONCLUSIONS

1. An influence of senility upon carbohydrate assimilation or diabetes is conceivable from a quantitative reduction or other alterations of metabolism, or from functional or anatomic changes in the pancreas. The observations upon senile dogs failed to show any departures from the normal in the glucose tolerance, the ratio of pancreas weight to body weight, the microscopic structure of the pancreas, the size of the pancreatic remnant with which diabetes occurs, the capacity of such a remnant for hypertrophy, or the clinical course of the diabetes.

2. An investigation was also made of the possible influence of the elevated metabolism or the pancreatic peculiarities of youth. Previous work had indicated that the glucose tolerance of puppies is less than that of adult dogs. No exact studies were made of the most important point in the microscopic anatomy, namely the richness in islands, but no striking departure from the adult average was noticeable in numerous routine observations. The ratio of pancreas weight to body weight was somewhat irregular, and the question of possible changes in an individual during growth could not be accurately decided, but the general average in puppies did not differ appreciably from that in adults, particularly with consideration of the small body weight. The remnant left after partial pancreatectomy generally grows considerably in puppies; the hypertrophy on the whole is greater than in adult dogs, but does not surpass what is found in occasional mature or adult animals, and may be slight or absent especially in older puppies. The tendency to diabetes is distinctly less in puppies than in adult dogs, partly on account of weakness and cachexia, partly because of hypertrophy of the pancreas remnant, and perhaps sometimes because of a high functional efficiency of a small remnant. There is no specific tendency to rapidity of downward progress or to diabetic acidosis in puppies.

3. An example is given of long delayed onset of diabetic symptoms on a fixed diet after gain of weight, this gain being largely growth instead of mere obesity. With a still milder diabetic tendency it is readily conceivable that the delay might be longer and might occur on an ordinary diet, also that the diet might in large measure determine the onset of symptoms. In this way it is possible that childhood infections injuring the pancreas may be responsible for some cases of diabetes which make their appearance at a much later period.

BIBLIOGRAPHY

(1) ALLEN: Amer. Journ. Med. Sci., paper 2 of this series (in press).
(2) ALLEN: Journ. Exper. Med., 1920, xxxi, 363.
(3) ALLEN: Amer. Journ. Med. Sci., paper 2 of this series (in press).
(4) ALLEN: Studies concerning glycosuria and diabetes, 1913, 905.
(5) ALLEN: Studies concerning glycosuria and diabetes, 1913, 34.
(6) PRATT: Journ. Amer. Med. Assoc., 1912, lix, 322.

EXPERIMENTAL STUDIES IN DIABETES

Series II. The Internal Pancreatic Function in Relation to Body Mass and Metabolism

9. The Influence of Pregnancy upon Experimental Diabetes

FREDERICK M. ALLEN

From the Hospital of The Rockefeller Institute for Medical Research, New York

Received for publication August 10, 1920

Diabetes may conceivably be aggravated by the increased metabolism of pregnancy, which involves both additional food assimilation and the formation of considerable new tissue. Unknown toxic or metabolic factors may possibly have an influence. An opposite possibility is suggested especially by the work of Carlson and collaborators (1), namely, that diabetes in the latter part of pregnancy may be prevented by the internal secretory activity of the fetal pancreas. The chief question here is whether internal secretions pass in appreciable quantities through the placenta. In clinical practice pregnancy has long been regarded as decidedly injurious, to such an extent that therapeutic abortion was commonly recommended in women suffering from any serious grade of diabetes. The most comprehensive and recent survey of clinical experience is by Joslin (2), who considers that the supposed injurious effects are largely explained by the higher diet taken. One case of apparent gain of tolerance during pregnancy (3) is inconclusive, partly because the blood sugar rose during gestation and partly because it is uncertain whether the changes observed were due strictly to the pregnancy.

Partially depancreatized animals offer the most accurate obtainable conditions for studying the effect of pregnancy upon the internal pancreatic function. Before proceeding to the principal experiments, it is desirable to mention briefly some controls and unsuccessful attempts relating to this problem.

One control to be thought of is the possible effect of changes in the sexual organs themselves upon the pancreas or its function. This is

part of the larger question of the interrelation of the sex glands with the pancreas and diabetes. A few extirpation experiments were performed as follows.[1]

Dog H2–89, a female mongrel aged 4 years, had been close to the verge of diabetes since the first operation on April 12, 1915, so that the removal of 0.25 gram additional pancreatic tissue on March 16, 1916 brought on mild diabetes. By repeated tests up to May 18 it was proved that glycosuria was regularly absent on a measured bread and soup diet, and was present to the amount of about 5 grams daily with the addition of 75 grams glucose. May 18, both ovaries were removed, together with the tubes and part of the uterine horns. A trace of glycosuria followed the operation, and another trace when the regular bread diet was fed. The addition of 75 grams glucose resulted in glycosuria of 12.5 grams one day and 13 grams another day. By May 27 glycosuria was again absent on the plain bread and soup diet and the glycosuria from glucose was no higher than before operation. The absence of any perceptible influence upon the diabetes, other than the transient effects of the trauma, was also confirmed by the animal's later history.

Dog D4–62. Female; mongrel; long haired; age 6 to 8 years; well nourished; weight 19.2 kilos. December 16, 1916, removal of pancreatic tissue weighing 27.5 grams. Remnant about main duct estimated at 3.2 grams ($\frac{1}{7}$ to $\frac{1}{6}$). After fasting, glycosuria was found consistently absent on a diet of 1 kilo of beef lung, and moderate (0.5 to 1 per cent) with addition of 50 grams bread. January 17, 1917, both ovaries were removed. A trace of glycosuria followed the operation, but ceased on the lung diet. With addition of 50 grams bread it was distinctly greater than before (2.8 to 4.8 per cent, in larger urine volumes), but ceased when the bread was omitted on January 26. Thereafter the diabetic tendency was no greater than before the oöphorectomy.

Dog C3–67. Male; Dalmatian; age 1½ years; in excellent nutrition; weight 17.6 kilos. March 14, 1916, castration, and removal of pancreatic tissue weighing 28.5 grams. Remnant about main duct estimated at 2.6 grams ($\frac{1}{14}$ to $\frac{1}{15}$). The dog was used for a few tests (especially feeding of pure fat or talcum powder, as described elsewhere) which had no influence upon the glycosuria. Otherwise no food was given, but heavy glycosuria continued until death occurred from weakness on April 1, at a weight of 11 kilos. The pancreas remnant, weighing 3.1 grams, was normal except for the usual vacuolation of island cells. The bladder urine contained 0.61 per cent of sugar, and the autopsy otherwise was negative. Such irrepressible glycosuria with a pancreas remnant of this size is so unusual that the possibility of an aggravating influence of the castration was suggested.

Dog D4–63. Male; mongrel; long haired; age 3 years; medium nutrition; weight 15.25 kilos. After removal of pancreatic tissue on February 14 and March 7, 1917, glycosuria was maintained almost continuously on bread and soup diet with addition of 200 grams glucose to March 23, after which it was absent. Such a duration of glycosuria is evidence that an animal is very close to diabetes, which will be produced by the removal of a trifle more pancreatic tissue. The actual absence of diabetes was confirmed by blood sugar tests, the plasma sugar being

[1] All operations were performed under ether anesthesia.

0.087 per cent before feeding and 0.145 per cent at the highest point afterward, and down to 0.122 per cent by evening even on the bread and glucose diet. March 28, castration was performed. Glycosuria remained absent on bread diet to April 2, and thereafter also with addition of 200 grams glucose. The attempts to induce diabetes by diet were continued through May, with merely the usual slight gain in tolerance. The removal of 0.5 gram pancreatic tissue on May 8 proved inadequate, but mild diabetes followed the removal of 0.65 gram additional on June 1.

This single experiment with dog DI-63 probably suffices to prove that removal of the testes has no influence for or against the production of diabetes. Aggravation of an existing diabetes, which is a separate question, was not produced by removal of the ovaries in dogs B2-89 and DI-62. The apparent aggravation after removal of the testes in dog C3-67 may have been purely accidental, and any specific influence remains improbable. Circumstances prevented further experiments of this sort.

Numerous attempts were made to determine the size of pancreas remnant with which diabetes occurs in pregnant animals, with uniform failure on account of abortion or death. Efforts to immunize by one or several preliminary subcutaneous or intraperitoneal injections of aqueous extract of fresh sterile dog pancreas gave no protection. Trials were made with removal of five-sixths to nine-tenths of the pancreas in single operations, performed as quickly and easily as possible so as to be over in half an hour or less, but the animals always became unwell and aborted within a few days or at most a week. Such an interval, especially when glycosuria is prevented by malaise and refusal of food, can decide nothing with the pancreas remnants mentioned, though permitting some observations after total pancreatectomy as in Carlson's experiments. Other attempts were made with successive operations, in the hope that each might be so easy as to avoid abortion, also by leaving a duodenal remnant and a subcutaneous graft early in pregnancy, with the idea that diabetes might be brought on later in pregnancy by simple removal of the graft. All such attempts failed for one cause or another. It is sometimes possible to remove considerable portions of pancreas, even in the later stages of pregnancy, without accident, just as other abdominal operations are often feasible; but any pancreatic resection to the point of diabetes seems absolutely incompatible with continuance of gestation. The possibility that this disturbance may be due to the sudden deficit of the internal pancreatic function was excluded by two sets of controls; first, the results are just as bad when the entire uncinate process is left as a subcutaneous graft in addition to the usual pancreatic remnant; second, pregnant

part of the larger question of the interrelation of the sex glands with the pancreas and diabetes. A few extirpation experiments were performed as follows.[1]

Dog B2–89, a female mongrel aged 4 years, had been close to the verge of diabetes since the first operation on April 12, 1915, so that the removal of 0.25 gram additional pancreatic tissue on March 16, 1916 brought on mild diabetes. By repeated tests up to May 18 it was proved that glycosuria was regularly absent on a measured bread and soup diet, and was present to the amount of about 5 grams daily with the addition of 75 grams glucose. May 18, both ovaries were removed, together with the tubes and part of the uterine horns. A trace of glycosuria followed the operation, and another trace when the regular bread diet was fed. The addition of 75 grams glucose resulted in glycosuria of 12.5 grams one day and 13 grams another day. By May 27 glycosuria was again absent on the plain bread and soup diet and the glycosuria from glucose was no higher than before operation. The absence of any perceptible influence upon the diabetes, other than the transient effects of the trauma, was also confirmed by the animal's later history.

Dog D4–62. Female; mongrel; long haired; age 6 to 8 years; well nourished; weight 19.2 kilos. December 16, 1916, removal of pancreatic tissue weighing 27.5 grams. Remnant about main duct estimated at 3.2 grams ($\frac{1}{1}\frac{1}{0}$). After fasting, glycosuria was found consistently absent on a diet of 1 kilo of beef lung, and moderate (0.5 to 1 per cent) with addition of 50 grams bread. January 17, 1917, both ovaries were removed. A trace of glycosuria followed the operation, but ceased on the lung diet. With addition of 50 grams bread it was distinctly greater than before (2.8 to 4.8 per cent, in larger urine volumes), but ceased when the bread was omitted on January 26. Thereafter the diabetic tendency was no greater than before the oöphorectomy.

Dog C3–67. Male; Dalmatian; age 1½ years; in excellent nutrition; weight 17.6 kilos. March 14, 1916, castration, and removal of pancreatic tissue weighing 28.5 grams. Remnant about main duct estimated at 2.6 grams ($\frac{1}{1}\frac{1}{4}$ to $\frac{1}{1}\frac{1}{5}$). The dog was used for a few tests (especially feeding of pure fat or talcum powder, as described elsewhere) which had no influence upon the glycosuria. Otherwise no food was given, but heavy glycosuria continued until death occurred from weakness on April 1, at a weight of 11 kilos. The pancreas remnant, weighing 3.1 grams, was normal except for the usual vacuolation of island cells. The bladder urine contained 0.61 per cent of sugar, and the autopsy otherwise was negative. Such irrepressible glycosuria with a pancreas remnant of this size is so unusual that the possibility of an aggravating influence of the castration was suggested.

Dog D4–63. Male; mongrel; long haired; age 3 years; medium nutrition; weight 15.25 kilos. After removal of pancreatic tissue on February 14 and March 7, 1917, glycosuria was maintained almost continuously on bread and soup diet with addition of 200 grams glucose to March 23, after which it was absent. Such a duration of glycosuria is evidence that an animal is very close to diabetes, which will be produced by the removal of a trifle more pancreatic tissue. The actual absence of diabetes was confirmed by blood sugar tests, the plasma sugar being

[1] All operations were performed under ether anesthesia.

0.087 per cent before feeding and 0.145 per cent at the highest point afterward, and down to 0.122 per cent by evening even on the bread and glucose diet. March 28, castration was performed. Glycosuria remained absent on bread diet to April 2, and therafter also with addition of 200 grams glucose. The attempts to induce diabetes by diet were continued through May, with merely the usual slight gain in tolerance. The removal of 0.5 gram pancreatic tissue on May 8 proved inadequate, but mild diabetes followed the removal of 0.65 gram additional on June 1.

This single experiment with dog D4-63 probably suffices to prove that removal of the testes has no influence for or against the production of diabetes. Aggravation of an existing diabetes, which is a separate question, was not produced by removal of the ovaries in dogs B2-89 and D4-62. The apparent aggravation after removal of the testes in dog C3-67 may have been purely accidental, and any specific influence remains improbable. Circumstances prevented further experiments of this sort.

Numerous attempts were made to determine the size of pancreas remnant with which diabetes occurs in pregnant animals, with uniform failure on account of abortion or death. Efforts to immunize by one or several preliminary subcutaneous or intraperitoneal injections of aqueous extract of fresh sterile dog pancreas gave no protection. Trials were made with removal of five-sixths to nine-tenths of the pancreas in single operations, performed as quickly and easily as possible so as to be over in half an hour or less, but the animals always became unwell and aborted within a few days or at most a week. Such an interval, especially when glycosuria is prevented by malaise and refusal of food, can decide nothing with the pancreas remnants mentioned, though permitting some observations after total pancreatectomy as in Carlson's experiments. Other attempts were made with successive operations, in the hope that each might be so easy as to avoid abortion, also by leaving a duodenal remnant and a subcutaneous graft early in pregnancy, with the idea that diabetes might be brought on later in pregnancy by simple removal of the graft. All such attempts failed for one cause or another. It is sometimes possible to remove considerable portions of pancreas, even in the later stages of pregnancy, without accident, just as other abdominal operations are often feasible; but any pancreatic resection to the point of diabetes seems absolutely incompatible with continuance of gestation. The possibility that this disturbance may be due to the sudden deficit of the internal pancreatic function was excluded by two sets of controls; first, the results are just as bad when the entire uncinate process is left as a subcutaneous graft in addition to the usual pancreatic remnant; second, pregnant

dogs ordinarily survive a week of fasting and phlorizination without abortion, though the glycosuria and acidosis apparently represent a greater disturbance of carbohydrate metabolism than that following the pancreatic operations mentioned.

An attempt to test the influence of the increased food requirement and lactose formation of the lactation period was made as follows.

Dog C3–90, mongrel, in excellent condition, weighing 20.5 kilos, was found pregnant in an operation on May 25, 1916, when the splenic process and body of the pancreas down to the main duct were removed, with the idea that diabetes might later be produced by a very easy operation for removal of the uncinate process. The pregnancy continued uneventfully, but by mistake too long an interval was allowed, and on June 22 the dog gave birth to eight healthy puppies. She was an excellent mother and the puppies all thrived until, on June 29, the uncinate process was removed in an operation requiring only a few minutes. There was quick recovery from the brief etherization, and the dog showed the usual devotion and nursed the puppies immediately on being returned to the cage. She acted well and lively but ate very little on the following days, during which time the pups continued to nurse though the mother paid less attention to them. By July 4 her appetite was fully restored, but she refused to have anything further to do with the pups, and even injured them if they approached to nurse. Part of the trouble may have been due to tenderness about the abdominal wound, but there seemed also to be a genuine breaking up of the physiology and psychology of lactation by the operation. Milk rapidly disappeared from the breasts. Diabetes remained absent notwithstanding glucose feeding. Whether the disturbances observed were peculiar to the pancreatic operation or might follow any other abdominal interference, the experience showed that this method was not applicable for studying the influence of lactation upon diabetes.

Numerous microscopic examinations have been made of the pancreas of dogs in various stages of pregnancy and of a few during lactation, without the finding of any departure from the normal in any respect.

It was evident that a satisfactory study was possible only through the occurrence of pregnancy in animals already diabetic, so as to reproduce exactly the conditions encountered in diabetic women. Properly prepared animals with potential diabetes are normal in their entire behavior, and only the unfavorable laboratory conditions made the experiment difficult. It was hoped that cats might be particularly suitable for the purpose, but in the only instance in which a partially depancreatized cat became pregnant the experiment ended in failure, as follows.

Cat A1–84. Female; strong adult; weight 3.5 kilos. December 20, 1913, pancreatic tissue was removed, not quite to the point of producing diabetes. The cat remained continuously free from glycosuria on meat, bread and milk diets

thereafter, but had a permanently lowered tolerance, as shown by the production of glycosuria in subcutaneous glucose tests by doses between 1 and 1.5 grams per kilo. Impregnation occurred February 18, 1914. Thereafter the diet was meat and milk, mixed with as much lactose as the animal could be induced to take. Considerable carbohydrate could thus be given, though cats usually object strongly to the sweetness of glucose or saccharose. About the middle of March the animal was noticed to be weak and unwell. Abortion occurred March 16 and death March 18. There was no diabetes and the pancreas remnant was normal. The fatal outcome was almost certainly a sugar intoxication (4), with no specific relation to the partial pancreatectomy or to diabetes. Cats may possibly prove to be well suited to pregnancy experiments in diabetes, but they are as a rule an unfavorable species for carbohydrate over-feeding.

Far more numerous attempts were made with partially depancreatized dogs during three years, but all failed owing to the unfavorable environment. Finally a successful experiment became possible in dog B2-00, which was particularly valuable for the purpose because of the long previous records which had established the tolerance accurately. These observations have been reported in previous papers, especially no. 3 of series I (5) and no. 1 of series II (6).

A series of operations beginning in 1913 had made the dog nearly diabetic, but after the removal of an additional 0.8 gram of pancreatic tissue on September 6, 1916, it was still impossible to maintain glycosuria with the heaviest bread and glucose feeding. Pregnancy then became evident, though the exact time of its beginning was unknown. Tests of the tolerance were performed by feeding, as described below. As diabetes remained absent with advancing pregnancy, there was danger that the entire result would be negative.

Accordingly, on December 16, 1916, 0.1 gram pancreatic tissue was removed, in an operation so short and easy that the dog did not even lose appetite for the day. Diabetes resulted, as proved by the fact that plain bread and soup feeding now maintained slight glycosuria (0.4 to 0.8 per cent, in 400 to 680 cc. urine). December 26 on this diet the plasma sugar was 0.091 per cent before feeding, 0.182 per cent four hours after. The special feeding test was repeated in late pregnancy on December 30.

As an additional experiment, it was attempted to learn whether the pregnant dog with latent diabetes had any special tendency to acidosis. Therefore nothing was fed on December 31, and only 300 grams suet daily on January 1, 2 and 3 (1917). Abortion then occurred, though the dog appeared only slightly unwell and remained free from acetone bodies in urine and blood, both before and afterward. The plasma sugar was constantly normal (0.089 to 0.105 per cent) after omission of carbohydrate, both before and after abortion. The carbohydrate tests had seemed harmless, and it may be possible that the fat feeding was responsible for the abortion even without acidosis.

January 27, the special feeding test was repeated as a control in the non-pregnant condition. The existence of diabetes was unmistakable. The diet was then changed to meat to prevent downward progress, and the dog was left most of the

Dog B2–00. Tests with feeding 100 grams beef lung, 200 grams bread and 150 grams glucose

DATE	WEIGHT	PLASMA SUGAR	Hb.	URINE		REMARKS
				Volume	Glucose	
1916	*kgm.*	*per cent*	*per cent*	*cc.*	*per cent*	
November 21	13.4	0.106	101	18	0	Before feeding
		0.123	97	16	0	2 hours after feeding
		0.156	98	24	Faint	4 hours after feeding
		0.128	96	38	Faint	6 hours after feeding
November 23		0.092	95		0	Before feeding same diet as Nov. 21
		0.095	88	13	Faint	2 hours after feeding
		0.139	92	No	urine	4 hours after feeding
		0.130	93	105	Faint	6 hours after feeding
December 16	Removed 0.1 gm. pancreatic tissue. Dog pregnant					
December 30	15.6	0.085			0	Before feeding
		0.345		212	3.45	2 hours after feeding
		0.455		192	6.46	4 hours after feeding
		0.500		68	5.89	6 hours after feeding
1917						
January 7	12.9	0.125		7	0	Before feeding. After abortion
		0.400		25	7.13	2 hours after feeding
		0.400		35	6.68	4 hours after feeding
		0.333		33	8.00	6 hours after feeding
February 23	16.3	0.116			0	Before feeding
		0.238		18	0.63	2 hours after feeding
		0.244		17	0.57	4 hours after feeding
		0.232		22	0.69	6 hours after feeding
May 17	15.6	0.130			0	Before feeding. (Early pregnancy)
		0.346		39	4.16	2 hours after feeding
		0.435		73	7.32	4 hours after feeding
		0.384		44	4.82	6 hours after feeding
July 11	14.9	0.106			0	Before feeding. (Late pregnancy.)
		0.322		35	4.72	2 hours after feeding
		0.304		65	3.95	4 hours after feeding
		0.312		68	5.58	6 hours after feeding
July 19	*13.0	0.077		0	0	Before feeding. (After delivery)
		0.200		23	0.72	2 hours after feeding
		0.218		16	0.92	4 hours after feeding
		0.232		13	0.95	6 hours after feeding

DATE	WEIGHT	PLASMA SUGAR	Hb.	URINE		REMARKS
				Volume	Glucose	
1917	*kgm.*	*per cent*	*per cent*	*cc.*	*per cent*	
August 9	12.9	0.081			0	Before feeding
		0.164		18	Very faint	2 hours after feeding
		0.109		36	Faint	4 hours after feeding
		0.135		17	Faint	6 hours after feeding
October 5	13.5	0.109			0	Before feeding
		0.159		17	0	2 hours after feeding
		0.145		36	Faint	4 hours after feeding
		0.152		46	0.31	6 hours after feeding
November 22	14.0	0.109			0	Before feeding
		0.204		24	Faint	2 hours after feeding
		0.238		36	0.39	4 hours after feeding
		0.125		24	0.44	6 hours after feeding

time with a male in the hope of a second pregnancy. The plasma sugar meanwhile remained normal (0.087 to 0.113 per cent) on the carbohydrate-free diet. The special feeding test was repeated on February 23 and May 17.

Pregnancy then became manifest, and the feeding test was repeated in this condition on July 11. June 28 and July 2, the plasma sugar on the meat diet was normal (between 0.075 per cent before feeding and 0.105 per cent after feeding).

July 12, less than 0.1 gram of pancreatic tissue was removed without appreciable disturbance.

Three pups were born on July 16. They were probably 2 or 3 days premature, and were all dead by July 19 owing to failure to nurse properly. This result may have been independent of the experimental conditions, for the dog was of a type which often has troubles with puppies.

The special feeding test was repeated on August 9, October 5 and November 22.

Owing to the perfect manner in which the dog bore the experiments through two pregnancies, the questions at issue received very satisfactory answers as follows:

a. In the first instance, pregnancy failed to produce diabetes in an animal which was so close to the verge that diabetes resulted from the removal of 0.1 gram additional pancreatic tissue. Against an assumption that the diabetes here was due largely to the pregnancy and only partially to the pancreas operation is the fact that diabetes persisted after termination of the pregnancy. Also in the second instance, when the dog was known to have mild diabetes but was free from symptoms on meat diet, pregnancy failed to produce either glycosuria or hyperglycemia on this diet.

b. The removal of a bit of pancreatic tissue in the latter part of each pregnancy supplemented the observations of the negative effects of gestation and especially demonstrated the absence of vacuolation of the islands first when the animal was not quite diabetic and second when she was mildly diabetic. The absence of such hydropic changes with latent diabetes corresponds fully to the experience in non-pregnant animals.

c. The passage of any appreciable quantity of pancreatic internal secretion from the fetuses to the mother is disproved by two facts. First, the occurrence of diabetes following the operation of December 16, 1916, was not prevented; in other words, there was no transfer of pancreatic hormone sufficient to compensate for the loss of 0.1 gram of maternal pancreatic tissue. Second, the feeding tests demonstrated an actual aggravation of the diabetes during pregnancy. Test meals were used instead of intravenous glucose injections because according to previous experience (7) they afford a more accurate index of the diabetic condition and also because they seemed to promise less danger of abortion or other accidents. Attention may be called to the results of these tests as shown in the table.

The test meal consisted of 100 grams beef lung, 200 grams bread and 150 grams glucose. Only slight hyperglycemia and faint glycosuria resulted from this diet on November 21 and 23, when the dog was non-diabetic. The removal of 0.1 gram pancreatic tissue on December 16 produced a radical change, so that the hyperglycemia and glycosuria in the test of December 30 were of plainly diabetic character. The tolerance was fully as low on January 7, after termination of pregnancy, but the dog was still unwell from the recent abortion. The test of February 23 showed a considerably better tolerance as judged by both blood and urine.

May 17, early in the second pregnancy, the test showed a well-marked fall in the assimilation, and the result was not greatly different in the late stage of pregnancy on July 11. The bit of pancreas removed on July 12 was so tiny that it apparently had little effect upon the tolerance. At any rate, in spite of this operation, the assimilation on July 19, three days after normal delivery, showed a decided improvement. This improvement was still greater on August 9, after all puerperal disturbance had subsided. It was also maintained up to October 5, the length of the experiment and the uniformity of results being thus sufficient to exclude accidental fluctuations of tolerance.

CONCLUSIONS

1. No positive influence of the sex glands upon diabetes was demonstrable by extirpation experiments. Also no anatomic changes in the pancreas were perceptible with pregnancy or lactation.

2. Observations upon a partially depancreatized dog during pregnancy are opposed to the view that any appreciable quantity of internal pancreatic secretion passes from the fetus to the mother.

3. A distinct lowering of carbohydrate assimilation was shown during pregnancy. This was not clearly associated with the increase of metabolism, in the sense either of increased food requirement or new tissue formation, for it seemed approximately the same in early and late pregnancy and was also evident during the illness following abortion. It may therefore be regarded chiefly as a toxic manifestation and thus classifiable with the influence of infection. The effect is relatively slight, because pregnancy failed to produce diabetes in a dog where the removal of 0.1 gram pancreatic tissue sufficed for the purpose, and also after the dog was demonstrably diabetic on carbohydrate diet pregnancy gave rise to neither glycosuria nor hyperglycemia on carbohydrate-free diet.

The tests with partial pancreatectomy, which affords the most exact method of study, suggest that Carlson's results in totally depancreatized dogs are to be interpreted as cachexia. Clearer information of the influence of pregnancy upon the internal pancreatic function is also afforded by the freedom from the variables which enter into clinical cases. The slight tendency to aggravation of the diabetes and the ready control by diet support Joslin's experience of the feasibility of completion of pregnancy by diabetic women under suitable conditions of treatment. If the toxic factor is the principal one, as suggested, the possibility remains that the injurious action in some women may be considerably greater than in dogs and accordingly may require more radical measures.

BIBLIOGRAPHY

(1) Carlson and Drennan: This Journal, 1911, xxviii, 391.
Carlson and Ginsburg: Ibid., 1914, xxxvi, 217.
(2) Joslin: Treatment of diabetes mellitus, 1917, 448.
(3) Joslin: Treatment of diabetes mellitus, 1917, 456.
(4) Allen: Journ. Exper. Med., 1920, xxxi, 393.
(5) Allen: Journ. Exper. Med., 1920, xxxi, 563.
(6) Allen: Amer. Journ. Med. Sci., 1920, clx, 781.
(7) Allen and Wishart: Journ. Biol. Chem., 1920, xlii, 415.

RHYTHMICITY OF THE PYLORIC SPHINCTER

HOMER WHEELON AND J. EARL THOMAS

From the Department of Physiology of the St. Louis University School of Medicine, St. Louis, Missouri

Received for publication August 19, 1920

The functions of the pyloric sphincter are usually considered dependent upon the physical condition and relative degree of acidity of the gastric content. Such an explanation fails in great part to account for certain phenomena associated with the processes of gastric evacuation both in health and disease. It also fails to consider the purpose of gastric motility save for mixing the gastric contents and discharging them into the duodenum at irregular periods of opening of the sphincter. However, recent work tends to show that the opening and closing of the sphincter is related, not only to the degree of fluidity and acidity of the gastric content, but also to peristalsis and the degree of tonicity demonstrated in the stomach.

Our present interest in the physiology of the sphincter was stimulated by our inability to explain, upon accepted theories, radiographic observations made upon the human stomach. We therefore undertook to study, upon laboratory animals under easily controllable conditions, the motility of the pyloric sphincter. In this study the term pyloric sphincter means that narrow band of muscle constituting the last portion of the stomach.

METHODS

Forty-four experiments were performed upon dogs in this series. All operative procedures were carried out under ether anesthesia. Unanesthetized dogs were studied radiographically. In 14 experiments records were obtained immediately following operation and while the animal was under the influence of a light anesthesia. Six dogs were studied from 5 to 18 hours after operation, no anesthesia being used during the taking of records. One animal was studied 3 days after operation, another after an interval of 2 weeks. Four animals were

operated under ether and morphine (10 mgm. per kilo) and studied at once, ether being discontinued during the observations. This procedure gave a quiet animal with a very active stomach. Although the type of sphincter action under this anesthesia was similar to that obtained under other anesthesias, it was discontinued because of the known action of morphine upon the gastro-intestinal tract. The most constant graphic results were obtained with only sufficient ether to abolish voluntary movements. Under such an anesthesia the behavior of the sphincter is more constant and uniform than in the unanesthetized animal under experimental conditions.

The operations in most cases consisted in opening the abdomen by a midline incision, entering the fundic portion of the stomach and sewing the recording apparatus in place. The gastric incision was then closed with a pursestring suture about the rubber tube leading from the anchored balloon. The opening in the abdomen was closed with a running suture. Aseptic precautions were observed in all cases in which the animal was permitted to survive 12 hours or longer. In 3 animals the muscle on either side of the pyloric sphincter was cut, thus confining action upon the apparatus to the sphincter proper.

All apparatus except the recording instruments was designed and constructed especially for this work. The apparatus is the equivalent of a balloon and is so constructed that it can be placed and maintained within the lumen of the pyloric sphincter. These balloons, or as we have termed them, *pylorographs*, are of three types: *a*, flexible, closed pylorograph; *b*, rigid, closed pylorograph, and *c*, open, flexible pylorograph.

The closed flexible pylorograph (fig. 1, *a*) consists of a short section of finger cot stretched over two cones of hard rubber the apices of which face each other. One cone is perforated by a small hole which leads out through a nipple on its base for the attachment of a rubber tube. When in position the rubber is pressed down upon the sloping surfaces of the cones by the tonus of the sphincter to such an extent that the recording apparatus is affected by the contraction of a width of muscle not exceeding 5 mm. This device when placed in position tends to remain there because of the V-shaped surfaces offered to the pyloric ring. Complete fixation of the pylorograph was obtained by passing a ligature around the base of each cone and securing it in the muscle.

The rigid pylorograph is similar to the one described above save that the two cones remain connected (fig. 1, *b*).

The open, flexible pylorograph (fig. 1, *c*) is constructed on the same principle as the flexible, closed pylorograph except that an open tunnel 4 mm. in diameter runs throughout its entire length. The apices of the two cones are connected by means of a piece of rubberdam tubing.

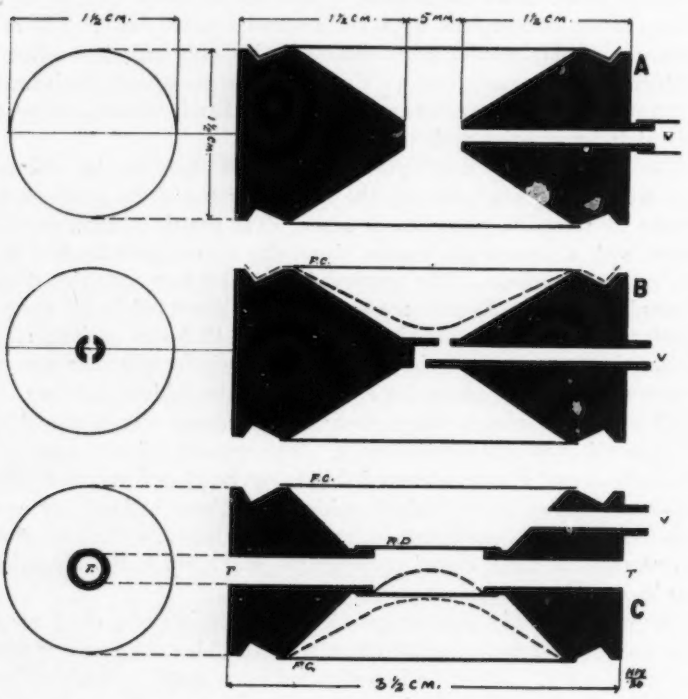

Fig. 1. Illustrations of the various types of pylorographs. *A*, Closed flexible pylorograph. *B*, Closed rigid pylorograph. *C*, Open flexible pylorograph. *V*, Air transmission vent for tube connection with water manometer. *F.C.*, Flexible wall of pylorograph made of finger cot. *T.*, Opening connecting stomach with duodenum. *R.D.*, Tube of rubber dam composing inner wall of air chamber. Dotted lines indicate positions of the rubber surfaces during contraction of the sphincter.

The bases are connected by a section of finger cot. This device, the equivalent of a tunnelled cylindrical balloon, permits the sphincter to demonstrate its motility and at the same time permits material to leave the stomach.

Graphic records were obtained with tambours, and piston and bellows recorders. The pylorograph was connected with the recording apparatus through a water manometer. The manometer was adjusted to a pressure (3 to 30 cm.) which gave best results in any given case. Air transmission was used throughout.

RESULTS

The pyloric sphincter demonstrates two types of motility; *a*, active rhythmic contractions (figs. 2 and 3), and *b*, tone waves (figs. 5 and 6). The characteristics of these two types of action are as follows.

a. Rhythmic contractions. The rhythmic movements of the sphincter are characterized by contractions and relaxations each followed by a quiescent period or pause (fig. 3). These contractions occur at the rate of from 3 to 5 per minute; that is, each cycle is completed in an interval of from 12 to 20 seconds. The phase of contraction is 4 to 5 seconds, the phase of relaxation 3 to 7 seconds; the quiescent phase plus the period of inhibition prior to contraction occupy the remainder of the cycle. These figures are necessarily only relative because of the different rates of contraction shown by various animals, the depth of the anesthesia, and also because of the general motility of the stomach and small intestines. During periods of rhythmic action of the sphincter the contractions and relaxations are uniform in degree; that is, the movements are initiated and consummated from a constant level (figs. 2 and 3). There is usually a definite degree of relaxation (inhibition) of tone immediately preceding a contraction. At times relaxation continues from the completion of contraction until the beginning of a second contraction, no definite phase of quiesence or inhibition being shown. Similar results were obtained both from the filled and recently emptied stomach.

The cycles of the sphincter are definitely altered under various conditions; also, periods of increased activity may occur; however, as will be shown in a later communication, such alterations bear a definite relation to anesthetics, trauma and the activities of the gastro-intestinal canal.

Rhythmicity of the sphincter is not lost following denervation of the stomach (fig 4).

b. Tonicity of the sphincter. The tonicity of the pyloric sphincter is gained or lost during a series of rhythmic contractions of which the heights of the individual contractions vary in proportion to the degree

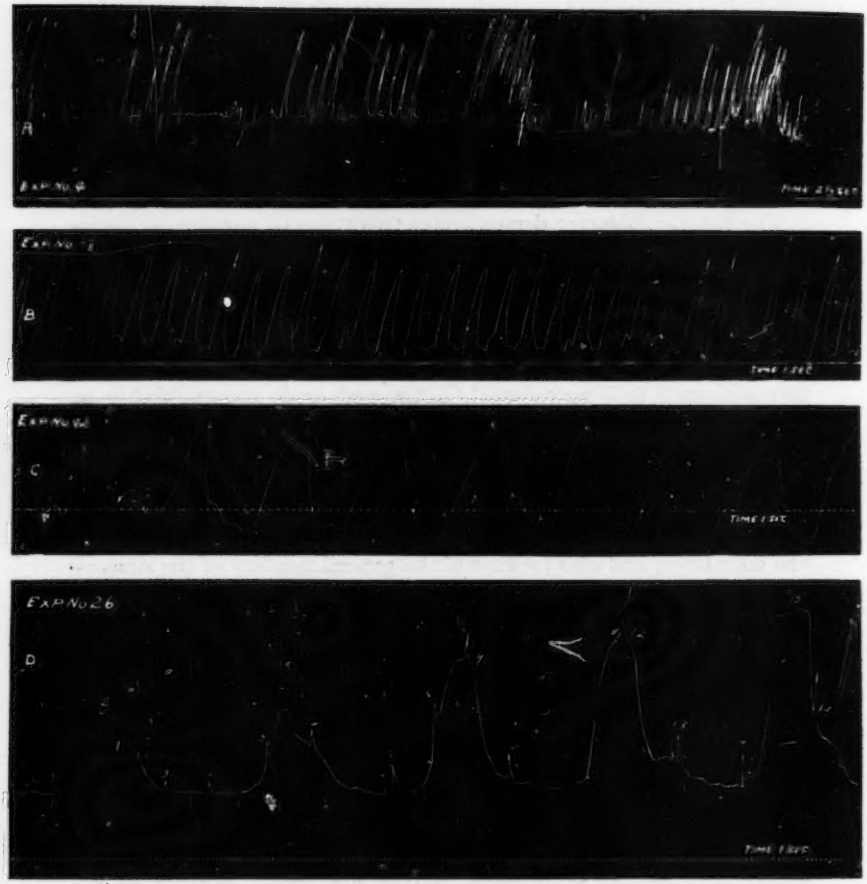

Fig. 2. Pylorograms obtained from four different experiments; *A*, experiment 4; *B*, experiment 19; *C*, experiment 42; *D*, experiment 26. Animals under ether anesthesia. Graph *A* obtained with tambour, *B* and *C* with piston recorder, *D* with bellows recorder. Time, 2½ seconds in trace *A*, 1 second in *B*, *C* and *D*.

Trace *A* shows periods of inactivity followed by rhythmic contractions and alterations in tonicity. Traces *B*, *C* and *D* demonstrate periods of constant rhythmic activity. Note the variation in the height of contractions in traces *A* and *D*. The four graphs are typical of the entire series of experiments.

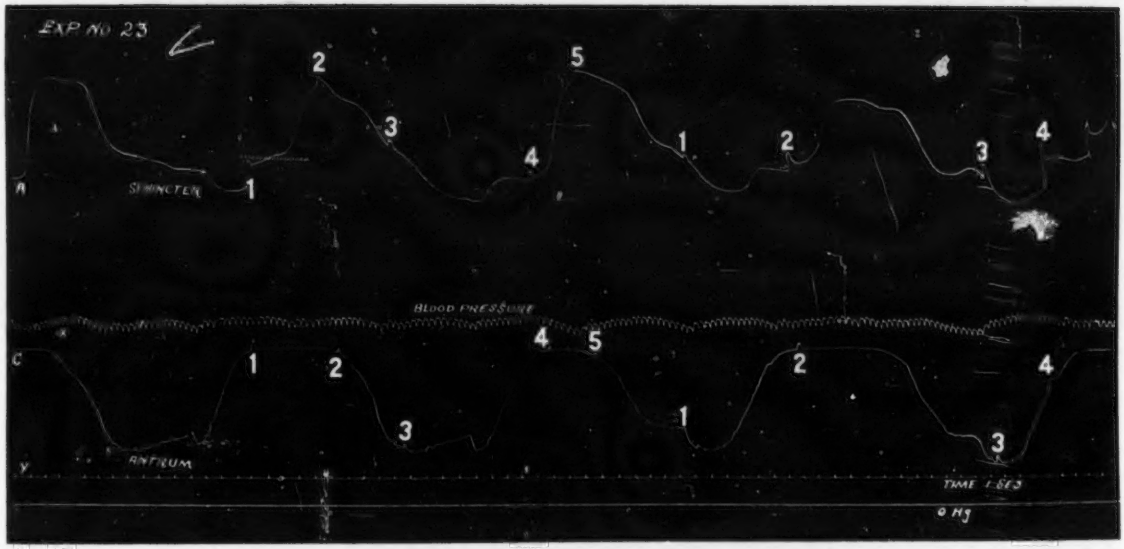

Fig. 3. Experiment 23; February 7 1920. Ether used throughout experiment. Records begun immediately following operation. Double rigid enterograph used. *A*, Sphincteric contractions. *X*, Blood pressure tracing *C*, Antral contractions. *Y*, Time in seconds. Numerals denote synchronous points.

Note that the antrum has reached its maximum at the beginning of the sphincter's contraction, the latter reaching its greatest degree of contraction just as the antrum begins to relax.

of tonicity gained (figs. 5 and 6). On the other hand, periods of increased or decreased rhythmic contractions may appear at a time of constant tonicity (fig. 5, trace *C*). Waves of increased tonicity appear to result because of a lengthening in the time required for complete relaxation; that is, a second wave of contraction appears during the relaxation phase of the previous cycle. A reduction in the degree of tonicity accompanies an increased degree of lengthening of the relaxation phase of the rhythmic cycles, the rate of the rhythmic contractions remaining constant.

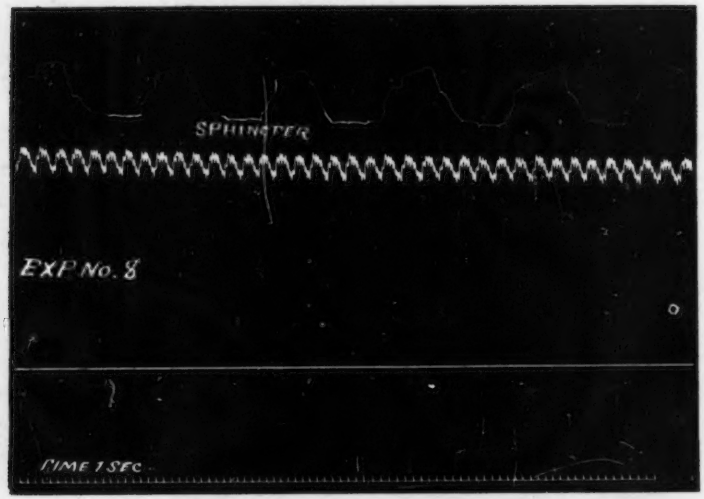

Fig. 4. Experiment 8; January 17, 1920. Rhythmic contractions of the pyloric sphincter following denervation of the stomach. Ether anesthesia. Time in seconds.

The rhythmicity and type of sphincter action are not altered by increasing or decreasing, within limits, the pressure within the pylorograph. However, if the pressure is greatly increased the height of the individual contractions is decreased. Longer excursions of the writing lever are recorded when the pressure in the pylorograph is low (3 to 10 cm. of water). In other words, the greater the degree of resistance offered by the pylorograph to the force of the sphincter's action the smaller becomes the excursion of the muscle while acting. The primary

effect of distention of the balloon in the pyloric canal is to excite the sphincter to rhythmic action. Following this there usually appears a gradual loss in tone for several moments after which the rhythmic contractions appear from a constant level. The rapidity with which the sphincter adapts its tonicity to an alteration in resistance is remark-

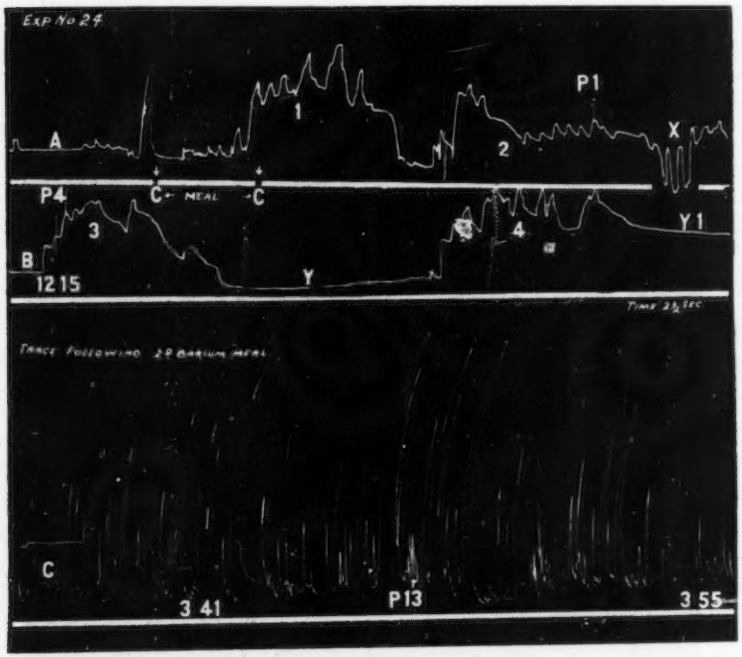

Fig. 5. Experiment 24; February 9, 1920. Pylorograms obtained by use of open pylorograph. Animal operated under ether. Records begun 18 hours later. Piston recorder used for registration. Experiment checked by radiographic records. *A*, *B* and *C*, Sphincter action. *C–C*, Administration of 8 ounces of barium mixture by stomach tube. *1*, *2*, *3* and *4*, Tone waves carrying contraction waves. Trace *C* shows three active and four reduced phases of activity.

able. Apparently, the normal tonicity is only sufficient to close the sphincter or to approximate its surfaces against those of a body in its lumen. Non-resisting materials permit of complete occlusion of the lumen during the positive phase of the sphincteric cycle. Tonicity

of the sphincter appears to be unusually high immediately following the ingestion of food, either normally or by means of the stomach tube. Tonicity is also high immediately following the opening of the abdominal cavity. Doubtless this is reflexly the result of peritoneal irritation. Tonicity is not lost by reason of operative procedures on the stomach.

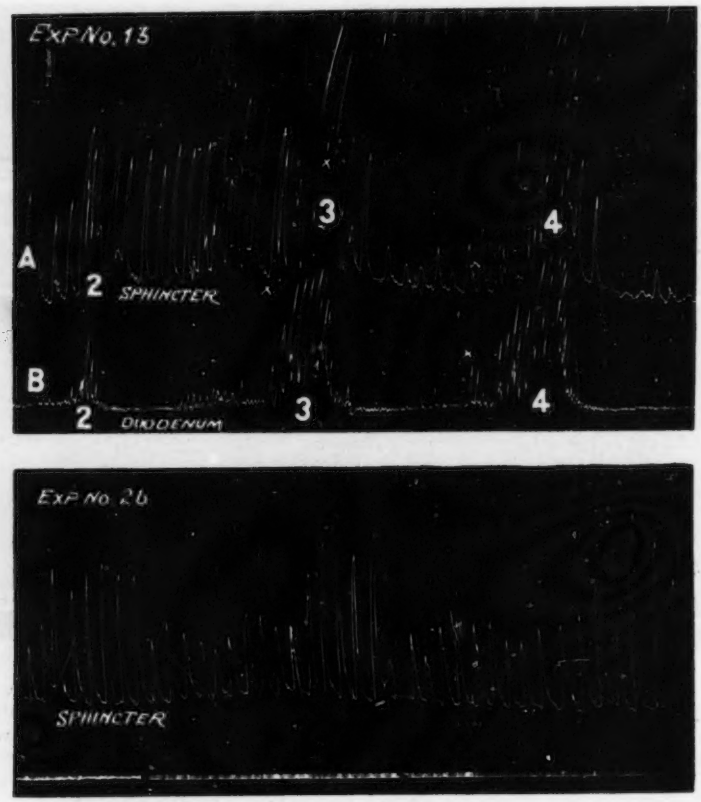

Fig. 6. Experiment 13; January 9, 1920. Ether anesthesia for operation. Double flexible enterograph. Records obtained 3 days after operation upon the conscious animal. *A*, Sphincter contractions. *B*, Duodenal contractions. Note periods of heightened tone of the sphincter at times of pronounced activity on the part of the duodenum (*3* and *4*). *X*, Synchronous points. Time in seconds.

The approach of the pylorograph or examining finger to the antral region causes marked contraction of the pars pylorica. Only under the influence of a deep or surgical anesthesia does the sphincter lose tone completely.

SPECIAL EXPERIMENTS

In dog 25 in which a permanent gastric fistula had been established, it was possible to insert the finger into the antrum and even through the pyloric canal into the duodenum without placing the animal under ether or causing it much discomfort. The primary effect of the finger in the antrum was to excite a state of violent and maintained contraction. Under a constantly applied pressure contracture passed off and the finger was finally permitted to pass through the sphincteric canal. With the finger in the canal one could distinctly feel pressure applied rhythmically during the positive phases of the sphincteric cycles. The sphincter never relaxed completely nor drew away from the finger during the relaxation phase or the period of quiescence. The observation that an initial irritation of the antrum causes a heightened degree of tonicity in the pars pylorica corroborates the generally accepted views as to the functions of these parts in relation to solid objects in the stomach.

In an effort to throw more light on the function of the sphincter the following experiment was performed. An open pylorograph (fig. 1, *c*), as described above, was anchored in the pyloric canal. Graphic records were begun 18 hours following operation (fig. 5). At this time the sphincter demonstrated powerful contractions which occurred irregularly. The animal was then placed on the radiographic table and given an 8-ounce meal of barium sulphate and milk by means of the stomach tube. Immediately following the withdrawal of the tube a marked tone wave appeared on the graphic tracing, superimposed upon which was a series of contractions of varying degree (fig. 5, *A-1*). This was followed by a series of smaller waves each of which lasted from 3 to 7 minutes. Four such tone waves are shown in tracings *A* and *B* of the same figure. As previously stated, these tone waves are built up during a series of rhythmic contractions and lost through a series of gradual and prolonged relaxations. At *X* in figure 5 a series of relaxations permitted the recording lever to drop below the tone level held by the sphincter prior to the administration of the meal. At points marked *Y* and *Y-1* there is a total absence of rhythmic contraction although the tonicity of the sphincter at *Y-1* is markedly above

that at *Y* where the level is lower than the pre-meal normal. A radiogram taken at the height of a rhythmic contraction (*P.1*), and at a time of relatively high tonicity, showed the antrum in a state of high tonicity with the pyloric canal closed. As time went on these severe tonic contractions gradually gave way to a constant tone level and rhythmic contractions of varying height (fig. 5, *c*). The stomach emptied itself of the barium mixture in less than 80 minutes. At the end of this time a second meal was given. Rapid distention of the operated stomach easily produces vomiting. This difficulty was overcome by using a small catheter as a stomach tube. The type of sphincter action shown in tracing *C* was continued for 4 hours, at the end of which time the stomach was completely free from the second meal.

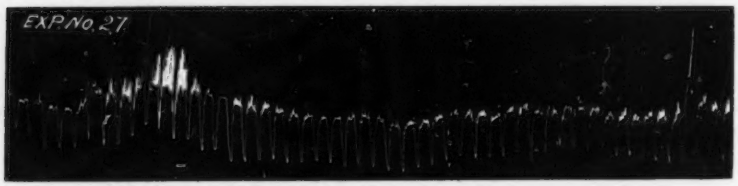

Fig. 7. Experiment 27; February 19, 1920. Ether used throughout experiment. Records begun immediately following operative procedures. Tambour myograph and piston recorder used to obtain graph. Because of construction of the myograph the contraction phase is recorded as the downstroke; relaxation the upstroke. Note the regularity of contraction and the tone level changes.

This experiment seems of special interest because it demonstrates that a foreign body in the pyloric canal through which the gastric contents may pass does not alter the normal progress of gastric evacuation. Further, it supplies information from both the experimental and the radiographic standpoint which indicates that the sphincter is rhythmical in its activities. The development of tone waves in this case does not differ in character from those observed by other types of recording apparatus through which the gastric contents may not pass.

A third type of experiment was performed to check the graphs obtained by means of the closed and open types of pylorographs. In these experiments, three in number, a tambour myograph was attached to the exterior of the sphincter, the animal being immersed in a tank of warm saline solution. The type of rhythmic contractions and tone

waves shown in figure 7 are common to the three experiments, and they show no fundamental variation from tracings obtained with other types of apparatus. The animal (no. 27) from which this figure was obtained had been fed about an hour before the experiment started. Often a gurgling noise was audible as material was forced from the antrum into the duodenum, these occurring during a wave of contraction of the pars pylorica.

The results obtained from the various types of experiments and radiographic studies are similar to each other, therefore the movements described for the pyloric sphincter may be considered as representative of normal functioning of this organ.

DISCUSSION

The closure of the pyloric sphincter, according to the prevailing theories, results because of: *a*, the presence of solid masses in the antrum which mechanically excite the mucosa of this region; *b*, an insufficient acidification of the gastric content; and *c*, the presence of acid chyme in the duodenum. Following liquefaction and acidification of the gastric contents these mechanical and chemical stimulants cease and the sphincter relaxes to permit the ejection of chyme into the duodenum.

The theory of an "acid control of the pylorus" (Cannon) does not account for the rapid discharge of water and solutions of neutral egg white from the stomach, neither does it explain the rapid clearance of the stomach in certain pathological conditions. In part the theory of fluidity accounts for the rapid evacuation of the stomach following the ingestion of fluid masses. However, this does not explain the processes involved in the control of the sphincter. Granting that the two theories do explain a certain number of facts relative to the control of the pylorus, we still have to account for pyloric activity when functioning in the absence of acid. That is, something more than fluidity and acid is necessary to open the sphincter to permit the passage of material into the duodenum. Granting that the sphincter is open, all theories agree that peristaltic contractions in the stomach are directly responsible for the discharge of gastric contents. Hence it may be assumed that intragastric pressure and peristalsis bear some definite relation to the activities of the sphincter. That posture and peristalsis do act in such a manner as to facilitate the emptying of the stomach has been pretty definitely shown.

Neilson and Lipsitz (5) have shown that posture to a great extent determines the time of retention of water in the stomach. Individuals lying on their right side retain less of a given amount of water at the end of a stipulated time than individuals assuming other positions. Cole (2), from a study of serial radiograms of the human stomach, has shown that the activities of the sphincter bear a definite relationship to the activities of the antrum in that the amount of contraction of the sphincter is in proportion to the activity of the gastric waves. He has also shown that during the active phase (contraction) of every gastric cycle the pyloric ring is open and a small portion of the gastric contents is propelled through its lumen into the reservoir cap. The terminal wave (peristaltic) which has meanwhile been advancing toward the sphincter, upon attaining it, effects its closure. The recent article by Luckhardt, Phillips and Carlson (4) clearly demonstrates that both in man and dogs the pyloric sphincter opens for the ejection of chyme with the arrival at the sphincter of powerful advancing rings of contraction and a general increase in the tone of the musculature of the stomach as a whole. Their observations demonstrate that a more definite relation exists between the muscular activity and the opening of the sphincter than between the opening of the sphincter and the reaction of the gastric contents. Ivy (3) made the suggestion prior to the appearance of the paper by Luckhardt, Phillips and Carlson, that the rhythmic discharge of water from the dog's stomach is such that it could very well correspond to peristaltic activity.

Such observations clearly demonstrate that the functions of the pyloric sphincter are dependent, in part at least, upon gastric motility. Our observations also tend to show that the sphincter because of its rhythmic type of motility acts in such a manner as to supplement gastric motility. Further observations to be reported later also show that the rhythmic contractions of the sphincter bear a definite and constant relation to the motility of the stomach (fig. 3).

SUMMARY AND CONCLUSION

1. A method is described for recording the motility of the pyloric sphincter.

2. The pyloric sphincter of the dog demonstrates rhythmic activity or cycles of motility which occur at the rate of from 3 to 5 per minute.

3. A single cycle of motility is characterized by a phase of contraction, relaxation and quiescence followed by a definite phase of inhibition prior to a subsequent contraction.

4. The sphincter demonstrates tone changes, such changes being gained or lost because of shortening or lengthening of the relaxation phase of the rhythmic cycles.

5. The observations here reported, therefore, show that the pyloric sphincter of the dog possesses the property of rhythmic contractility, the degree of which is influenced because of changes in tonicity.

BIBLIOGRAPHY

(1) CANNON: This Journal, 1898, i, 359; The mechanical factors of digestion, Longmans, Green and Co., New York, 1911; This Journal, 1907, xx, 283.
(2) COLE: Journ. Amer. Med. Assoc., 1913, lxi, 762; This Journal, 1916, xlii, 618.
(3) IVY: This Journal, 1918, xlvi, 420.
(4) LUCKHARDT, PHILLIPS AND CARLSON: This Journal, 1919, l, 57
(5) NEILSON AND LIPSITZ: Journ. Amer. Med. Assoc., 1915, lxiv, 1052.

A DIFFERENCE BETWEEN THE MECHANISM OF HYPERGLYCEMIA PRODUCTION BY ETHER AND BY CHLOROFORM

ELLISON L. ROSS AND L. H. DAVIS

From the Department of Physiology and Pharmacology, Northwestern University Medical School

Received for publication August 21, 1920

It has already been reported that there are grounds for believing that the hyperglycemia produced by ether anesthesia is due chiefly to the action of ether to reduce the influence of the internal secretion of the pancreas (1). It is considered that this reduction of the action of the internal secretion of the pancreas is a reduction of the inhibitor influence on glycolysis. Since the primary source of blood dextrose is liver glycogen, any injury to the liver should affect the ease with which glycogen is set free. Whether the injury to the liver cells should make the liberation of dextrose easier or more difficult there is no way to foretell. It is well known that chloroform is capable of producing liver pathology. Davis and Whipple (2) showed that chloroform anesthesia produced injury to liver cells and this injury could be increased by fasting before the administration of chloroform. It was thought worth while to compare the hyperglycemia from ether and from chloroform with special reference to the injury produced in the liver.

Experimental work. A group of five normal undieted dogs was given ether for half an hour. Anesthesia was induced by inserting the animal's head into a cylinder into which air that had passed through ether was forced. The animals were bled before anesthesia and after fifteen minutes of anesthesia. The blood sugar was determined by Benedict's method (3). The next day the same procedure was repeated except that the animals were kept under the anesthetic only fifteen minutes. The results are given in tables 1 and 2.

A second group of dogs was given chloroform to the surgical anesthetic stage and kept at this degree of anesthesia for half an hour and the following day the response to fifteen minutes of ether anesthesia was determined. The bleeding, analyses and administration of the anesthetic were carried out as before. The results are given in table 3.

TABLE 1

Ether hyperglycemia of a group of normal dogs

ANIMAL	GLYCEMIA BEFORE ETHER	GLYCEMIA AFTER 15 MINUTES ETHER ANESTHESIA	INCREASE
1	0.113	0.143	0.030
2	0.084	0.111	0.027
3	0.116	0.127	0.011
4	0.096	0.141	0.045
5	0.105	0.132	0.027
Average.....	0.1028	0.1308	0.028

TABLE 2

Ether hyperglycemia of same group of dogs, day following half an hour ether

ANIMAL	GLYCEMIA BEFORE ETHER	GLYCEMIA AFTER 15 MINUTES ETHER ANESTHESIA	INCREASE
1	0.100	0.166	0.066
2	0.093	0.106	0.013
3	0.117	0.140	0.023
4	0.097	0.129	0.032
5	0.085	0.144	0.049
Average.....	0.1004	0.1370	0.0366

TABLE 3

Ether hyperglycemia one day following half hour chloroform anesthesia

ANIMAL	GLYCEMIA BEFORE ETHER	GLYCEMIA AFTER 15 MINUTES ETHER ANESTHESIA	INCREASE
6	0.095	0.106	0.011
7	0.092	0.116	0.024
8	0.099	0.120	0.021
9	0.097	0.136	0.039
10	0.097	0.106	0.009
Average.....	0.0960	0.1168	0.0208

TABLE 4

Ether hyperglycemia following half hour chloroform anesthesia after fast

ANIMAL	GLYCEMIA BEFORE ETHER	GLYCEMIA AFTER 15 MINUTES ETHER ANESTHESIA	INCREASE
11	0.094	0.097	0.003
12	0.093	0.134	0.041
13	0.088	0.094	0.006
14	0.094	0.116	0.022
15	0.095	0.115	0.020
Average.....	0.0928	0.1112	0.0184

A third group of animals fasted two days. Then these dogs were put through the same treatment as the second group. The results are given in table 4.

Discussion. When a drug is administered repeatedly the later reactions usually differ in degree from the first one. This may be due to decreased or increased sensitiveness of nerves, glands or tissue, or injury to cells. To determine whether or not ether had less power to produce hyperglycemia the second day than the first, the first two series of tests were made.

According to tables 1 and 2, the average glycemia before ether the first day was 0.1028 and the second day 0.1004 per cent. This small difference is negligible. Half an hour of ether anesthesia did not change the amount of blood sugar found the following day. This harmonizes with the general impression that ether anesthesia causes very little if any injury to the subject. The first day fifteen minutes of ether anesthesia brought the glycemia up to 0.1308 per cent and the following day the same procedure produced an average glycemia of 0.1370 per cent. The average increase the first day was 0.028 per cent and the average increase the next day was 0.0366 per cent. There was apparently an increased tendency for ether to liberate dextrose the day following half an hour of ether anesthesia. The reason for this increase we do not attempt to give at this time. However, we are able to make the important deduction that ether did not injure the mechanism involved in the production of ether hyperglycemia. If such an injury had occurred the reaction to ether the second day would have been less than that of the first day and the normal glycemia before anesthesia the second day would have been less than that of the first day.

The effect of chloroform on the glycemia of the following day is shown in tables 3 and 4. The average blood sugars before ether of the first and second groups of animals on the day following the chloroform anesthesias were 0.096 and 0.0928 per cent respectively. These values compared with those of normal dogs shown in tables 1 and 2—0.1028 and 0.1004 per cent—indicate a decided tendency of chloroform to partially paralyze the mechanism of sugar mobilization. This phase of the action of chloroform is of great importance. In view of the conclusion of Cannon that blood sugar is the most satisfactory source of energy in emergency, it is of the greatest importance that the mobilization of blood dextrose be not interfered with at a time such as that following an operation when often every vital function is strained to the limit in order to sustain life.

Fifteen minutes of ether anesthesia the day following a chloroform anesthesia of half an hour produced an average glycemia of 0.1168 per cent for the group of non-fasting dogs and a glycemia of 0.1112 per cent for the group of fasting animals. These glycemias compared with those of the group of normal dogs (0.1308 per cent) and of the group which had an ether anesthesia of half an hour the preceding day (0.1370 per cent), show a decided inability of the animals given chloroform the previous day to develop as great a hyperglycemia as normal animals.

The increases in blood sugar due to fifteen minutes of ether anesthesia of the groups of animals which received a chloroform anesthesia the preceding day, were 0.0208 and 0.0184 per cent. The average increase due to ether anesthesia of untreated dogs given in table 1 was 0.028 per cent and the average increase of a group of 17 dogs, given in a previous publication (4), was 0.037 per cent. A comparison of these increases from ether anesthesia obtained the day following a chloroform anesthesia with the increases of normal dogs shows a considerable decrease in the power of the animals to mobilize dextrose after chloroform anesthesia.

There were two groups of animals that were given ether the day following chloroform anesthesia of half an hour. These animals differed in that the second group fasted two days before the tests were made. The day following chloroform anesthesia the non-fasting animals had an average glycemia of 0.0960 per cent and the fasted animals a glycemia of 0.0928 per cent. Fifteen minutes of ether anesthesia of unfasted dogs produced an average increase of blood dextrose of 0.0208 per cent, making a glycemia of 0.1168 per cent. A similar ether anesthesia of the fasting animals produced an increase of 0.0184 per cent making the average blood dextrose value 0.1112 per cent. A comparison of the normal glycemias, the increases due to fifteen minutes of ether anesthesia, and the glycemias after the ether anesthesia of the group of fasting animals and of the group of non-fasting animals, shows greater values for blood dextrose in every case for the non-fasting group. These differences are small but constantly in favor of the one group.

SUMMARY AND CONCLUSIONS

A group of dogs was anesthetized with ether for half an hour. The next day the dogs were anesthetized with the same drug for fifteen minutes. The blood sugar changes were measured for the first fifteen minutes of anesthesia both times.

A second group of dogs was anesthetized with chloroform for half an hour and the following day each was given fifteen minutes of ether anesthesia. The blood dextrose changes the second day were measured.

A third group of animals fasted two days and was then treated the same as the second group.

Half an hour of ether anesthesia did not alter the glycemia of the following day, and did not decrease the hyperglycemia resulting from fifteen minutes of ether anesthesia.

Half an hour of chloroform anesthesia produced on the following day a glycemia lower than normal, an increase in blood dextrose due to fifteen minutes of anesthesia less than normal, and a hyperglycemia from fifteen minutes of ether anesthesia lower than normal.

A fast of two days preceding half an hour of chloroform anesthesia produced on the following day a still lower glycemia and still less reaction to fifteen minutes of ether anesthesia than occurred in non-fasting dogs.

These results in conjunction with the conclusions of Davis and Whipple (2) that the liver injury produced by chloroform is increased by a fast preceding anesthesia leads us to the following conclusions:

1. Ether anesthesia does not produce any injury to the mechanism of dextrose mobilization that can be detected the following day.

2. The injury to the liver cells produced by chloroform anesthesia reduces the glycemia of the following day and injures the mechanism of dextrose mobilization according to the degree of injury.

3. The hyperglycemia due to chloroform anesthesia is not due primarily to the direct action of chloroform on the liver. Probably chloroform, like ether, produces hyperglycemia chiefly through its depressing action on the internal secretion of the pancreas.

BIBLIOGRAPHY

(1) Ross and Davis: This Journal, 1920, liii, 391.
(2) Davis and Whipple: Arch. Int. Med., 1919, xxiii, 612.
(3) Benedict: Journ. Biol. Chem., 1918, xxxiv, 203.
(4) Ross: Journ. Pharm. Exper. Therap., 1919, xii, 377.

DIGESTIBILITY OF SOME HYDROGENATED OILS

ARTHUR D. HOLMES AND HARRY J. DEUEL, JR.

From the Office of Home Economics, U. S. Department of Agriculture[2]

Received for publication August 28, 1920

Until quite recent times table and culinary fats used in the United States were obtained almost wholly from the animal kingdom—dairy butter being the universal table fat and lard the principal culinary fat. The constantly decreasing per capita supply of animal fats has caused a very rapid increase in the use of vegetable oils for food, until olive, cottonseed, peanut and corn oils are now more or less generally used not only for salad but also for cooking purposes. Except for salad purposes, in the past the housewife apparently preferred fats which were very nearly, if not actually, solid at ordinary temperatures. To meet this demand for solid fats, vegetable oils were hardened either by removing a portion of their low melting constituents or by the addition of stearin or a fat rich in stearin, and fats prepared by one or the other of these methods came into quite general use under a variety of trade names. These processes for hardening vegetable oils have now been largely replaced by the hydrogenation process which is based on the discovery that hydrogen may be added, under proper conditions of temperature and pressure, to the glycerides of the unsaturated fatty acids by means of a catalytic agent, such as nickel in a finely divided state. When the process is carefully controlled it is possible to prepare hydrogenated oils having any desired melting point.

Since it is well known that some metals when taken into the alimentary tract under certain conditions are toxic, it has been very properly questioned whether the ingestion of hydrogenated oils containing appreciable amounts of a metallic catalyst might not be followed by harmful physiological disturbances. Recent investigations (1) indicate that properly prepared hydrogenated oils do not contain sufficient nickel to produce toxic effects. The Federal Meat Inspection Division (2) shares

[1] Published with permission of the Secretary of Agriculture.

[2] Prepared under the direction of C. F. Langworthy, Chief, Office of Home Economics.

this opinion and permits the sale for edible purposes of those hardened oils which do not contain over two parts of the catalyzer per million parts of oil—an amount about five times that ordinarily found in normal hydrogenated oils produced in this country.

The literature contains relatively little information regarding the digestibility of hydrogenated oils. Thoms and Muller (3) found that a hardened oil melting below body temperature was more satisfactory than one in which more complete saturation occurred and a harder fat obtained. Smith, Miller and Hawk (4) determined the relative digestibility of lard (melting at 45°C.) and hydrogenated cottonseed oil (melting at 36°C.) and found the lard to be 94.7 per cent and the hardened oil 93.4 per cent digested—a difference, which in the opinion of the authors, is well within the limits of experimental error. Pekelharing and Schut (5) found that on a diet which contained no fat other than hydrogenated cottonseed oil, mice maintained a steady body increment. They also determined the digestibility of some hydrogenated cottonseed oils in feeding experiments on a dog. On the average the dog digested about 90 per cent of the hydrogenated oils and increased its weight by about one-third the original weight. From the results of the four months' experiment they found that the digestibility of the hydrogenated oils was inversely proportional to their melting points, that mixtures of lard and hardened oils were more completely digested than the hardened oils alone, that no physiological disturbances occurred, and that the feces of the hydrogenated oil diets contained more fat and more fatty acids than those resulting from the lard diet. Erlandsen, Fridricia and Elgstrom (6) report studies of hardened whale oil in which the digestibility varied from 91.6 per cent to 94.9 per cent for butter and whale oil; the difference in digestibility of the two fats did not exceed 0.9 per cent.

The present paper reports a series of digestion experiments with hydrogenated cottonseed, peanut and corn oils in which the entire sample was subjected to the hydrogenation process. Material is also available for reporting the results of experiments on the digestibility of blended hydrogenated oils, in which some hydrogenated oil, hardened to a high melting point, was mixed with enough untreated oil to give a fat of the desired hardness. This investigation is a continuation of an extended series of digestion experiments which has been conducted by this office with about fifty more or less common animal and vegetable fats. The general conclusions from these studies are that edible fats are highly digestible, that they do not unfavorably influence the digestibility of

other constituents of the diet, and that when eaten in normal amounts they do not cause any pronounced laxative effects.

Experimental. In these experiments the hydrogenated oils under consideration were included in the diet by being incorporated in the usual specially prepared cornstarch blancmange (7) or pudding. The basal ration, which was nearly fat-free, consisted of wheat biscuit, fruit, sugar and clear tea or coffee. The subjects of the experiments reported below were young men, apparently in normal health, from 20 to 40 years old, students in local universities, who as a result of their previous experience in this type of studies were familiar with the experimental procedure and who were thoroughly trustworthy. The experimental methods for separation and collection of feces and analysis of foods and feces outlined in earlier papers (8) were followed in the experiments below.

The presence of ether-soluble metabolic products in the feces has been taken into account and a suitable correction introduced in calculating results. Since the feces of a given day do not necessarily represent the residue of the food for the preceding day, it has seemed best in these experiments as in all the preceding ones to identify the feces of the experimental period with charcoal or carmine markers and to retain all the feces belonging to the period for analysis. Since the experimental procedure has been uniform throughout the tests on the animal and vegetable fats, the results obtained in these experiments are directly comparable with each other and with the results of our earlier studies.

Results of studies of the digestibility of common fats and oils indicate that their digestibility varies inversely with their melting points in the case of those melting above body temperature. In order to study the relationship between the digestibility and melting points of hydrogenated oils, those considered here were so chosen that some melted above and some below the temperature of the body (37°C.).

The majority of the samples of hydrogenated oil were hardened in the laboratory of Carleton Ellis by one of us (H. J. D.). J. R. Kuhn, of that laboratory, assisted in their preparation.

The melting points of the hydrogenated cottonseed oil were 35°C., 38.6°C. and 46°C.; the melting points of hydrogenated peanut oil were 37°C., 39°C., 43°C., 50°C. and 52.4°C.; and the melting points of hydrogenated corn oil were 33°C., 43°C. and 50°C. The iodine numbers of the samples are given in table 4.

All the hydrogenated oils included in this study were of a white color, solid or practically so at ordinary room temperature, and without any

characteristic odor or flavor. When melted they were of a straw yellow color resembling melted tallow. They were very homogeneous and if heated sufficiently they boiled without any sputtering and did not smoke until a relatively high temperature was reached. On cooling, in some instances, different portions of the resulting mass differed slightly in physical appearance, quite likely because of a partial separation of the softer and harder constituents of the hydrogenated oils.

The experiments made with the hydrogenated corn, cottonseed and peanut oils were carried out under conditions essentially the same as those of experiments with the same kinds of oil untreated. As earlier reports show, those oils had coefficients of digestibility as follows: corn oil (9), 96.9 per cent; cottonseed oil (10), 97.8 per cent and peanut oil (11), 98.3 per cent.

Digestibility of hydrogenated corn oil. Fifteen digestion experiments were conducted with hydrogenated corn oil, five each with hardened fats having a melting point of 33°C., 43°C. and 50°C. These fats were prepared by one of the authors (H. J. D.) at a large commercial research laboratory and are believed to be typical of commercial hardened corn oil.

The same group of subjects assisted in each series of experiments with hydrogenated corn oils and the usual standardized experimental conditions were employed.

This report of the individual experiments with hydrogenated corn oil and other oils included in this investigation is somewhat condensed, but the experimental data in full are on file in the Office of Home Economics, States Relations Service, U. S. Department of Agriculture.

The data which were obtained from the study of hydrogenated corn oils are summarized in table 1.

The average amount of hydrogenated corn oil eaten per man per day was 78 grams for the fat melting at 33°C., 74 grams for the 43°C. fat, and 44 grams for the 50°C. fat. The digestibility of the hardened corn oils studied was for 33°C. oil, 94.7 per cent and for 43°C. oil, 95.4 per cent; thus from the standpoint of practical dietetics there was no material difference in the digestibility of these oils. The digestibility of hydrogenated corn oil melting at 50°C. was 88.5 per cent which is identical with that of mutton fat (12) (88 per cent) melting at 50°C. The coefficients of digestibility obtained in these experiments are somewhat lower than 96.8 per cent (13) reported in an earlier paper for the digestibility of commercial, edible corn oil.

The experimental diet as a whole was well utilized, the carbohydrates being very completely absorbed, which would indicate that hydrogenated corn oils having melting points a very little higher than body temperature did not have any unfavorable effect on the digestibility of the other constituents of the diet.

TABLE 1

Summary of digestion experiments with hydrogenated corn oil in a simple mixed diet

EXPERIMENT NUMBER	MELTING POINT OF HYDROGENATED CORN OIL	SUBJECT	DIGESTIBILITY OF ENTIRE RATION			DIGESTIBILITY OF HYDROGENATED CORN OIL
			Protein	Fat	Carbohydrate	
	°C.		*per cent*	*per cent*	*per cent*	*per cent*
1022	33	W. V. D.	75.3	90.8	97.8	93.5
1023	33	H. L. G.	71.0	93.6	96.8	96.7
1024	33	E. L. M.	69.6	91.5	97.4	94.5
1025	33	G. S. M.	72.2	87.6	98.1	90.7
1026	33	J. C. W.	72.0	95.0	96.9	97.9
Average			72.0	91.7	97.4	94.7
1037	42	W. V. D.	83.0	92.5	98.3	95.0
1038	42	H. L. G.	81.0	93.8	97.1	96.8
1039	42	E. L. M.	70.3	89.4	96.6	93.6
1040	42	G. S. M.	70.5	92.5	97.3	96.9
1041	42	J. C. W.	76.6	90.7	95.9	94.6
Average			76.3	91.8	97.0	95.4
1042	50	W. V. D.	65.2	84.8	97.8	90.2
1043	50	H. L. G.	65.9	81.6	95.9	87.9
1044	50	E. L. M.	86.2	91.2	98.3	94.0
1045	50	G. S. M.	60.7	78.9	97.5	85.6
1046	50	J. C. W.	69.9	79.4	97.0	84.9
Average			69.6	83.2	97.3	88.5

Digestibility of hydrogenated cottonseed oils. The experiments made with hydrogenated cottonseed oil were conducted under experimental conditions identical with those employed in the study of the digestibility of cottonseed oil which was found to be 97.8 per cent digested (14). The hydrogenated oils which have received attention in this investigation were not all prepared from the same lot of cottonseed oil; one lot melting at 35°C. (used in experiment 512) was a well-known commercial product which was purchased in the open market; the fat melting at 38.6°C. was specially prepared for our studies in the research laboratories

of a concern manufacturing edible hydrogenated oils; the fats melting at 35°C. (used in experiments 1027–1031) and 46°C. were prepared by one of us in a large consulting laboratory. While these products were not all of commercial origin it is believed that they are, nevertheless, typical of the commercial article.

Table 2 summarizes the data from the experiments with hydrogenated cottonseed oil.

TABLE 2

Summary of digestion experiments with hydrogenated cottonseed oil in a simple mixed diet

EXPERIMENT NUMBER	MELTING POINT OF HYDROGENATED COTTONSEED OIL	SUBJECT	DIGESTIBILITY OF ENTIRE RATION			DIGESTIBILITY OF HYDROGENATED COTTONSEED OIL
			Protein	Fat	Carbohydrate	
	°C.		*per cent*	*per cent*	*per cent*	*per cent*
512	35	P. K.	67.0	92.6	96.6	96.2
1027	35	W. V. D.	84.8	96.9	99.0	98.3
1028	35	H. L. G.	61.5	94.4	95.2	98.6
1029	35	E. L. M.	70.2	94.4	97.9	97.0
1031	35	J. C. W.	63.6	89.7	95.7	93.9
Average			69.2	93.6	96.9	96.8
459	38.6	R. L. S.	69.5	92.7	97.3	95.5
1052	46	W. V. D.	75.9	93.8	98.4	96.3
1053	46	H. L. G.	69.5	93.2	96.7	96.4
1054	46	E. L. M.	68.7	91.0	97.7	91.9
Average			71.7	92.7	97.6	94.9

The average digestibility of the entire ration in the above experiments indicates that this diet was quite well digested. The daily consumption of hydrogenated oil in these experiments was on the average about 84 grams. In the last group of experiments with hydrogenated cottonseed oil melting at 46°C., 89 grams of the fat were eaten daily without causing any physiological disturbances, which would indicate that the limit of tolerance for this fat was in excess of 89 grams. The coefficients of digestibility, 96.8 per cent for hydrogenated oil having a melting point of 35°C., 95.5 per cent for hydrogenated oil having a melting point of 38.6°C. and 94.9 per cent for hydrogenated oil having a melting point of 46°C., indicate that the hydrogenated cottonseed oils having these melting points are well utilized by the body.

Digestibility of hydrogenated peanut oil. Twenty digestion experiments were made with hydrogenated peanut oils having melting points of 37°C., 39°C., 43°C., 50°C. and 52.4°C. under the usual uniform experimental conditions. The peanut oils melting at 39°C. and 52.4°C. were prepared for us through the courtesy of the chief chemist of a large manufacturing concern. The oils melting at 37°C., 43°C. and 50°C. were hydrogenated by one of us at a commercial fat and oil research laboratory. The results of the experiments with hydrogenated peanut oil are given in table 3.

The average amounts of hydrogenated peanut oil eaten daily in the above groups of experiments were: 37°C. fat, 76 grams; 39°C. fat, 78 grams; 43°C. fat, 91 grams; 50°C. fat, 59 grams; and 52.4°C. fat, 62 grams. No instance of intestinal disturbance resulted which indicates that the above quantities of these hardened oils are well tolerated by the average adult.

A comparison of the melting points and digestibility of these hardened oils is of interest. An increase of 2 degrees in the melting point (from 37°C. to 39°C.) was accompanied by a 2 per cent decrease in digestibility, an increase of 4 degrees from 39°C. to 43°C. caused no significant change in digestibility, an increase of 7 degrees from 43°C. to 50°C. caused a decrease of 4.5 per cent in digestibility, while an increase of 2.4 degrees from 50°C. to 52.4°C. caused a decrease of 13 per cent in digestibility. The coefficient of digestibility of 79 per cent for peanut oil melting at 52.4°C. is the lowest value obtained for any fat of this series; the digestibility (15) of mutton fat (melting point 50°C.) being 88 per cent and that of oleo stearin (16), 80 per cent. Since the melting point, 52.4°C., of this hardened oil is considerably higher than 37°C., the temperature of the human body, it is probable that in the process of digestion saponification takes place only on the exterior of the particles of hardened oil which decrease in size as the process of digestion continues. If surface area be thus a factor, then the rate of digestion and possibly the extent of digestion of a hydrogenated fat having a high melting point is governed to some extent by the size of the particles of hydrogenated oil ingested. Additional experiments are necessary to supply conclusive evidence on this point.

The hydrogenated oil melting at 37°C. was as completely digested (98.1 per cent) as the untreated peanut oil which was found to be 98.3 per cent absorbed (17) by the body. As a group the hydrogenated peanut oils were well digested and well tolerated by the subjects of this investigation.

TABLE 3

Summary of digestion experiments with hydrogenated peanut oil in a simple mixed diet

EXPERIMENT NUMBER	MELTING POINT OF HYDROGENATED PEANUT OIL	SUBJECT	DIGESTIBILITY OF ENTIRE RATION			DIGESTIBILITY OF HYDROGENATED PEANUT OIL
			Protein	Fat	Carbohydrate	
	°C.		*per cent*	*per cent*	*per cent*	*per cent*
1012	37	W. V. D.	74.7	95.1	97.5	97.6
1013	37	H. L. G.	78.3	96.9	97.5	99.1
1014	37	E. L. M.	68.3	94.3	97.4	97.4
1015	37	G. S. M.	57.7	94.0	96.3	97.9
1016	37	J. C. W.	66.7	94.9	95.8	98.6
Average			69.1	95.0	96.9	98.1
464	39	H. R. G.	74.0	95.4	98.1	97.8
466	39	P. K.	81.3	94.2	97.8	96.3
467	39	R. L. S.	66.7	90.2	96.8	93.7
Average			74.0	93.3	97.6	95.9
1032	43	W. V. D.	87.0	95.9	98.3	97.6
1033	43	H. L. G.	71.0	93.7	95.0	97.5
1034	43	E. L. M.	74.6	91.1	97.2	94.1
1035	43	G. S. M.	51.6	90.1	96.8	94.7
1036	43	J. C. W.	84.6	96.5	96.9	98.6
Average			73.8	93.5	96.8	96.5
1057	50	W. V. D.	70.0	87.0	98.2	90.6
1058	50	H. L. G.	65.4	88.5	96.1	93.1
1059	50	E. L. M.	69.9	90.3	97.8	93.6
1060	50	G. S. M.	69.0	86.6	98.1	90.7
Average			68.6	88.1	97.6	92.0
472	52.4	D. D. G.	65.0	65.8	96.4	71.6
473	52.4	H. R. G.	61.0	73.7	96.3	79.5
475	52.4	P. K.	41.7	81.8	98.9	85.9
Average			55.9	73.8	97.2	79.0

SUMMARY

Three vegetable oils, cottonseed, peanut and corn, have been partially hydrogenated to obtain hardened oils having different melting points and their digestibility studied. The results are summarized in table 4.

With the exception of peanut oil melting at 52.4°C. and the corn oil melting at 50°C. all of the hydrogenated oils were 92.0 per cent or more digested. They did not cause any observed digestive disturbances nor did they decrease the digestibility of the experimental diet as a whole. In general, the results showed uniformly that as the melting point of the oil was increased the coefficient of digestibility was decreased. The

TABLE 4

Summary of digestion experiments with hydrogenated vegetable oils in a simple diet

KIND OF HYDROGENATED VEGETABLE OIL	MELTING POINT OF HYDROGENATED VEGETABLE OILS	IODINE NUMBER	NUMBER OF EXPERIMENTS	DIGESTIBILITY OF ENTIRE RATION			DIGESTIBILITY OF HYDROGENATED VEGETABLE OILS
				Protein	Fat	Carbohydrate	
	°C.			*per cent*	*per cent*	*per cent*	*per cent*
Cottonseed	35	89.6	5	69.2	93.6	96.9	96.8
	38.6		1	69.5	92.7	97.3	95.5
	46	72.8	3	71.7	92.7	97.6	94.9
Peanut	37	81.3	5	69.1	95.0	96.9	98.1
	39		3	74.0	93.3	97.6	95.9
	43	78.8	5	73.8	93.5	96.8	96.5
	50	58.5	4	68.6	88.1	97.6	92.0
	52.4		3	55.9	73.8	97.2	79.0
Corn	33	89.0	5	72.0	91.7	97.4	94.7
	43	74.9	5	76.3	91.8	97.0	95.4
	50	55.4	5	69.6	83.2	97.3	88.5

coefficient of digestibility decreased at a much faster rate in the case of fats with melting points above 46°. The number of experiments conducted with the majority of the hydrogenated oils under consideration, it is believed, is sufficient to permit of fairly definite conclusions regarding the digestibility of each of the oils in question.

The results of these experiments indicate that these hydrogenated oils are as well utilized as natural fats of corresponding melting points.

BIBLIOGRAPHY

(1) SUFSMANN: Arch. Hyg., 1915, lxxxiv, 121.
LACKEY AND SAYRE: Journ. Amer. Pharm. Assoc., 1917, vi, 348.
LEHMANN: Chem. Ztg., 1914, xxxviii, 798.
(2) KERR: Personal communication.
(3) THOMS AND MULLER: Arch. Hyg., 1915, lxxxiv, 54.

(4) Smith, Miller and Hawk: Journ. Biol. Chem., 1915, xxiii, 505.
(5) Pekelharing and Schut: Pharm. Weeklad., 1916, liii, 769.
(6) Erlandsen, Fridricia and Elgstrom: Tidskr. Kem., 1918, xv, 109.
(7) Langworthy and Holmes: U. S. Dept. Agric. Bull. 310, 1915.
(8) Langworthy and Holmes: U. S. Dept. Agric. Bull. 310, 1915.
Langworthy and Holmes: U. S. Dept. Agric. Bull. 505, 1917.
Langworthy and Holmes: U. S. Dept. Agric. Bull. 507, 1917.
Holmes: U. S. Dept. Agric. Bull. 613, 1919.
Holmes: U. S. Dept. Agric. Bull. 630, 1918.
Holmes: U. S. Dept. Agric. Bull. 687, 1918.
Holmes: U. S. Dept. Agric. Bull. 781, 1919.
Deuel and Holmes: Journ. Biol. Chem., 1920, xli, 227.
(9) Holmes: U. S. Dept. Agric. Bull. 687, 1918, 20.
(10) Langworthy and Holmes: U. S. Dept. Agric. Bull. 505, 1917, 18.
(11) Langworthy and Holmes: Ibid.
(12) Langworthy and Holmes: U. S. Dept. Agric. Bull. 310, 1915, 21.
(13) Holmes: U. S. Dept. Agric. Bull. 687, 1918, 20.
(14) Langworthy and Holmes: U. S. Dept. Agric. Bull. 505, 1917, 18.
(15) Langworthy and Holmes: U. S. Dept. Agric. Bull. 310, 1915, 21.
(16) Holmes: U. S. Dept. Agric. Bull. 613, 1919, 16.
(17) Langworthy and Holmes: U. S. Dept. Agric. Bull. 505, 1917, 18.